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Effect of Heat–Moisture Treatment on Crystallinity, Digestibility Properties, Bioactive Compounds, and Antioxidant Activity of Purple Rice (*Oryza sativa* L. *indica*) Flour

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Abstract: Purple rice flour was modified using heat–moisture treatment (HMT) in three cycles using an oven (OHMT) and autoclave (AHMT) at temperatures of 100 °C and 120 °C, and with moisture levels of 20%, 25%, and 30%. X-ray diffraction was used to analyze the changes in the molecular structure. The swelling capacity, solubility, and starch digestibility, including rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS), were studied in both native and modified purple rice flour. the bioactive compounds and antioxidant activities were also evaluated. Both OHMT and AHMT resulted in a decrease in swelling capacity, solubility, and RDS, but an increase in RS and SDS values compared to the native purple rice flour. All samples showed an increase in relative crystallinity. Both treatments also had an impact on the bioactive compounds and antioxidant activities, leading to a decrease in total phenolic content, total anthocyanin content, and the scavenging activity of DPPH and ABTS radicals compared to the native purple rice flour. The findings suggest that HMT can both improve the functional properties of purple rice flour and hold potential for use in various food industries.

Keywords: purple rice flour; heat–moisture treatment; digestibility; swelling capacity; solubility; antioxidant activity

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1. Introduction

Purple rice (*Oryza sativa* L. *indica*), which is mainly grown in Southeast Asia and is a variety of colored rice, is known for its antioxidant properties [1,2] as well as its hepatoprotective [3], anti-diabetic [4], and anti-inflammatory effects [5]. Previous research has explored the chemical composition and biological effects of purple rice, but there is a lack of information on modifying purple rice to increase its value and enhance the stability of its antioxidant properties in the digestive system. Therefore, changes in physicochemical and functional qualities are required to increase its economic value [6–10]. Heat–moisture treatment (HMT), a cost-effective and straightforward physical modification method, has been shown to greatly affect the physical and chemical properties of starch or flour [9,11–20]. For HMT, a physical change occurs at a temperature higher than the glass transition temperature (Tg) but lower than the gelatinization temperature, lasting between 15 min and 16 h [21–23].

Native starch contains both amylose and amylopectin and can be classified into three groups based on its digestion rate and completeness. Rapidly digestible starch (RDS) refers to the amount of starch that enzymes can digest and enter the bloodstream within 20 min of digestion, while slowly digestible starch (SDS) refers to the amount of starch that can be fully digested within 20–120 min, as defined by Englyst and Cummings in 1986 [24]. Resistant starch (RS) is a type of starch that is not digested in the small intestine within 120 min and is classified as the third type of starch. It passes through to the large intestine intact and is fermented by gut bacteria, which produce short-chain fatty acids that have a range of health benefits [25,26]. There are five sub-types of RS, namely RS1, RS2, RS3, RS4, and RS5, each with distinct examples. RS1 is present in whole or partly milled grains, seeds, and legumes such as oats, brown rice, and beans. Raw potatoes, green bananas, and unripe plantains are examples of RS2. RS3 is formed by the retrogradation of starch in cooked and cooled potatoes, pasta, and bread. RS4 is found in chemically modified starches used in processed foods. RS5 is not commonly found in foods, but it can be produced under controlled conditions by heating a mixture of amylose and lipids [27]. Research has demonstrated that consumption of RS has multiple distinctive physiological functions, such as the regulation of gut microbiota, improved cholesterol metabolism, and overall metabolism. Moreover, it has been shown that RS intake decreases the likelihood of developing colon cancer, cardiovascular disease, diabetes, and other colon cancer-related conditions [28,29]. RS is a popular raw material in the food industry due to its resemblance to dietary fiber, health benefits, and high thermal stability. Furthermore, RS remains stable during most food processing procedures with minimal impact on food texture and taste [30]. The focus of research has shifted towards extracting RS from various sources and incorporating it into foods as a low-carbohydrate component [29,31].

The effect of hydrothermal processes on purple rice were recently improved using HMT treatment [32]. Furthermore, increases in RS and SDS content have been observed in various forms of starch including sweet potatoes [12], tartary buckwheat [17], wheat [33], and Japonica broken rice [34]. The impact of HMT was more apparent on the paste, gel, and thermal characteristics of rice flour with a moisture content between 20 and 30% [35]. The chemical changes that occur during the HMT process in flour are similar to those in starch because the protein and fat constituents of flour interact with the starch molecules to create a greater network [20,36]. Heating can induce denaturation of proteins that are present in a starch system, leading to the formation of a complex between lipids and amylose on the starch granule surface, which may inhibit the swelling and gelatinization of the granules [15].

The potential function of HMT modification on purple rice flour, despite its high carbohydrate content, has not been widely studied or reported. HMT has been shown to have an impact on flour or starch production when used alone or in combination with annealing and a ball milling process [37]. When applied to cassava, rice, and pinhao starch, a dual HMT process (involving an autoclave) was found to have a substantial impact on crystallinity, swelling power, and solubility when compared to single HMT [13,20]. The starches derived from corn, pea, lentil, and navy beans underwent hydrothermal treatment using either single or dual methods. Subsequently, these modified starches were annealed (ANN) and treated with HMT [22,38]. The research indicated that using HMT, ANN–HMT, or HMT–ANN techniques improved the thermal stability of waxy rice starch more effectively than using ANN alone. However, the combined use of hydrothermal treatments (ANN–HMT and HMT–ANN) led to increased levels of RS content compared to the unmodified starch [20,39].

There has been evidence that the cycles of HMT modification can alter the physicochemical properties of a variety of starch sources [14,20,40]. Type-A starches are dissimilar as they have a high degree of crystallinity and are not easily broken down by digestive enzymes [41,42]. To improve the functional properties of purple rice flour by increasing its crystallinity and rendering it less susceptible to digestive enzymes, this research aimed to investigate the impact of HMT on RS by varying the moisture content, temperature, and thermal source (oven and autoclave). A confirmation of impacts was achieved through identification and appropriate examination of swelling power, solubility, crystallinity, and changes in the antioxidant potential in the HMT process and digestibility properties. These results are valuable to the food industry as they allow for the development of more functional and nutritious food products.

2. Materials and Methods

2.1. Materials

Purple rice (*Oryza sativa* L. *indica*) grown in Mae Chaem District, Chiang Mai Province, Thailand, and the reagents, including D-glucose assay kit (GOPOD Format) were purchased from Megazyme International Ireland ltd., Ireland), α-amylase (22 U/mg), and amyloglucosidase (300 U/mL), Folin–Ciocalteu reagent, gallic acid, trolox, and 2,2-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical as well as other necessary materials were obtained from Sigma-Aldrich (St. Louis, MO, USA). All other reagents were of analytical grade.

2.2. Chemical Compositions of Purple Rice Flour

Association of Official Analytical Chemists (AOAC) methods [43] were employed for proximate analysis (moisture, ash, protein, and fat). In addition, the carbohydrate content was calculated as the percentage remaining after measuring all other components.

2.3. Preparation of Purple Rice Flour

The purple rice grains underwent grinding using a grinder, followed by passage through a 100 μm sieve, after which the resultant purple rice flour was stored in opaque and hermetically sealed containers at 4 °C.

2.4. HMT Treatment in Three Cycles

The present study employed two methods of heat–moisture treatment (HMT), namely oven heat–moisture treatment (OHMT) and autoclave heat–moisture treatment (AHMT), which were performed with specific modifications to the procedures outlined by Klein et al. [13] and Asranudin et al. [20] with certain modifications. Accordingly, 35 g of purple rice flour with 20%, 25%, and 30% moisture content were placed in a 250 mL Duran glass container, which was then sealed and stored at a temperature of −4 °C for a period of 24 h.

The OHMT cycle used in this study comprised heating the samples separately in an oven (Minimex MMO48L1 48L) at T_1 = 100 °C and T_2 = 120 °C for a 1 h. The sample was cooled to room temperature after heating, and then it was equilibrated at −4 °C for 24 h. Each treatment was repeated for 3 cycles, and the samples containing water contents of 20%, 25%, and 30% were labeled as $OHMT1_{20}$, $OHMT1_{25}$, $OHMT1_{30}$, $OHMT2_{20}$, $OHMT2_{25}$, and $OHMT2_{30}$, respectively.

In the AHMT process, the Duran glass container was heated at 100 °C and 120 °C using an autoclave (Sanyo MLS-3780) for a duration of 1 h with a loose cap. After heating, the sample was cooled to room temperature, and subsequently, it was equilibrated at −4 °C for 24 h. The experiment was repeated three times and yielded the samples $AHMT1_{20}$, $AHMT1_{25}$, $AHMT1_{30}$, $AHMT2_{20}$, $AHMT2_{25}$, and $AHMT2_{30}$.

The symbols 1 and 2 were used to represent HMT values of 100 °C and 120 °C, respectively.

2.5. Swelling Capacities and Solubilities

According to the procedure outlined by Su et al. [44] and Asranudin et al. [20] with slight modifications, 500 mg of sample was placed in a tube with 20 mL of distilled water. The mixture was soaked in a water bath (Memmert WNB 45) at 90 °C for 30 min, cooled to room temperature, and then centrifuged for 15 min at 3000× *g*. The resulting supernatant was decanted and subjected to evaporation for 5 h at 110 °C. The weight and amount of the dried precipitate were then recorded, and the produced residue was used to calculate the amount of starch dissolved in water. The solubility (%) and swelling capacity were also determined by analyzing the wet and dry mass of the supernatant and precipitate (g/g) using the provided Equations (1) and (2).

$$\text{Swelling capacity (g/g)} = \text{Weight of the wet mass sediment (g)}/\text{Weight of sample (g)} \quad (1)$$

$$\text{Solubility (\%)} = \text{Weight of dry supernatant (g)}/\text{Weight of sample(g)} \times 100 \quad (2)$$

2.6. Determination of Starch Fractions

The method of Englyst et al. [45] and Babu et al. [46] was utilized to analyze the starch fractions in the samples. A 200 mg starch sample was hydrolyzed with an enzyme solution containing porcine pancreatic α-amylase (290 U/mL) and amyloglucosidase (15 U/mL), where U represents the amount of enzyme that produces 1.0 mg of glucose from starch in 1 min at 37 °C and pH 5.2. The conical tubes containing the starch samples (200 mg) were mixed with a phosphate buffer (0.2 mol/L, pH 5.2, 15.0 mL), and the enzyme solution (5.0 mL) was added to the sample tube after a 5 min equilibration at 37 °C, followed by incubation in a water bath at 37 °C with shaking. The enzyme activity was stopped by collecting 0.5 mL aliquots at 20- and 120 min intervals, which were then mixed with 80% ethanol (4.0 mL) and centrifuged at 2000× g for 10 min. The total glucose concentrations of the 20- and 120-minute hydrolysate (G_{20} and G_{120}, respectively) were determined using a D-glucose assay kit (GOPOD Format Cat. No. K-GLUC; Megazyme International Ireland ltd., Ireland) with a measuring range of 4 to 100 µg of glucose per assay. The percentages of rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) in the samples were calculated using Equations (3)–(5) provided below:

$$\text{RDS} = G_{20} \times 0.9 \quad (3)$$

$$\text{SDS} = (G_{120} - G_{20}) \times 0.9 \quad (4)$$

$$\text{RS} = 100 - \text{RDS} - \text{SDS} \quad (5)$$

2.7. X-ray Diffraction (XRD)

X-ray diffraction was used to characterize native and modified purple rice flour in accordance with Asranudin et al. [20]. A 1.0 g sample was irradiated with CuKα radiation using a Rigaku MiniFlex II Benchtop X-ray Diffractometer and the scanning range of the angles (2θ) was from 5° to 45°. The relative degree of crystallinity (RC) was determined using 8.5 Origin (Microbial, Northampton, MA, USA) and calculated using Equation (6) developed by Nara and Komiya [47].

$$\text{Relative crystallinity (\%)} = A_c/A_c + A_a \times 100 \quad (6)$$

where A_c and A_a correspondingly represent the crystalline and amorphous areas.

2.8. Measurement of Phenolic Content and Anthocyanin Content

The Folin–Ciocalteu assay was used to measure the total phenolic content (TPC) following the method by Swain and Hillis [48] and Yea et al. [49]. A sample 0.10 g was treated with 20.0 mL of 75% methanol and subjected to centrifugation at 1500× g for 10 min. Then, 1.0 mL of the resulting extract or a solution of gallic acid with a concentration ranging from 50 to 150 mg/L was mixed with 5.0 mL of Folin–Ciocalteu's reagent, which had been previously diluted with water in a 1:10 ratio. The mixture was left to react for 3 min, followed by the addition of 4.0 mL of a sodium carbonate solution (7.5%, w/v). The resulting mixture was left to stand for 2 h. The TPC was determined by measuring the absorbance at 760 nm and expressed in mg GAE/g of the sample.

To determine the total anthocyanin content (TAC), two previously reported methods with slight modifications were employed as described by Burgos et al. [50], Yea et al. [49] and Ahmed et al. [51]. Briefly, a 1.0 g sample was mixed with 15.0 mL of a methanol solution containing 75% methanol and 1.0 M hydrochloric acid. The resulting mixture was stirred in the dark for 3 h and subjected to centrifugation at 1500× *g* for 10 min. The supernatant was then analyzed for its absorbance at 535 nm using a spectrophotometer (Thermo Scientific, GENESYS 20). TAC was expressed as cyanidin-3-glucoside equivalents per grams of dry weight by calculating it using Equation (7):

$$TAC = (A \times MW \times DF)/\varepsilon \times W \times 100 \quad (7)$$

where A = absorbance, MW = molecular weight of cyanidin-3-glucoside chloride ($C_{21}H_{21}ClO_{11}$, 484.84 Da), DF = dilution factor, ε = molar absorptivity (34,300), and W = sample mass (g).

2.9. Antioxidant Activities

The DPPH radical scavenging activity was assessed using the method outlined by Brand-Williams et al. [52] and Yea et al. [49]. A sample weighing 0.10 g was mixed with 3.0 mL of methanol and subjected to centrifugation at 660× *g* for 5 min. The resulting supernatant (0.1 mL) or a Trolox solution is a reference compound used to measure the antioxidant activity. In this experiment, concentrations ranging from 0.005 to 0.045 mg were mixed with 3.9 mL of an 80 µM methanolic DPPH solution and left to react for 3 h before measuring the absorbance at 517 nm. The results were calculated using the following Equation (8):

$$\text{DPPH radical scavenging capacity (\%)} = (A_c - A_s)/A_c \times 100 \quad (8)$$

where A_c represents the absorbance of control, and A_s represents the absorbance of the samples. The amount of DPPH radical scavenging capacity in the sample is expressed as mg Trolox equivalent per gram of sample (mg TE/g).

The ABTS radical scavenging activity was carried out following the method of Li et al. [53] and Chen et al. [54]. The ABTS solution was prepared by mixing 5 mL of ABTS with 88 µL of 140 mM potassium persulfate and incubating in the dark at room temperature overnight, followed by adjusting to an absorbance of 0.7 at 734 nm. Then, the sample was mixed with the ABTS reagent in a 1:10 ratio and incubated in the dark at room temperature for 30 min. The absorbance was measured at 734 nm using a spectrophotometer, and the ABTS radical scavenging capacity was calculated using a specific Equation (9):

$$\text{ABTS radical scavenging capacity (\%)} = (A_c - A_s)/A_c \times 100 \quad (9)$$

where A_c represents the absorbance of control, and A_s represents the absorbance of the samples. The amount of ABTS radical scavenging capacity in the sample is expressed as mg Trolox equivalent per gram of sample (mg TE/g).

2.10. Granule Morphology

The granule morphology of the samples was observed using a scanning electron microscope (JSM 5910 LV) following the methodology of Ashwar et al. [55] at magnifications of 5000× and 8000×. The samples were placed on double-sided sticky tape on aluminum stubs and were coated with a layer of gold–palladium.

2.11. Statistical Analysis

The data were analyzed by performing an analysis of variance and subsequently using Duncan's multiple range test. Pearson's test (two-tailed) was used to examine the correlation between the parameters. SPSS (version 22.0, IBM Corp., Armonk, NY, USA.) was employed for all statistical analyses.

3. Results and Discussion

3.1. Chemical Compositions of Purple Rice Flour

Table 1 presents the chemical composition of purple rice flour including the contents of protein, fat, ash, and carbohydrate, which are 8.01%, 0.32%, 0.42%, and 80.82%, respectively.

Previous studies have found that rice flour typically contains 71–91% starch, 7–11% protein, 0.87–8.1% lipids, and 0.46–1.1% ash [56,57]. Chemical composition dissimilarities in flour and starch can lead to variations in their susceptibility to HMT, as well as their paste and gel properties [35].

Table 1. Chemical compositions of purple rice flour.

Sample	Chemical Composition (%)				
	Moisture	Protein	Fat	Ash	Carbohydrate
Purple rice flour	10.44 ± 0.15	8.01 ± 0.09	0.32 ± 0.03	0.42 ± 0.12	80.82 ± 0.32

The presented data are expressed as means ± standard deviations.

A high protein content affects specific characteristics of modified flour. Furthermore, when exposed to heat, the proteins undergo denaturation and create a layer on the exterior of the starch granules. This layer hinders the granules' ability to absorb water and dissolve [15]. The presence of protein and fiber may hinder the activity of enzymes and cause an increase in SDS [41]. A high concentration of amylose promotes retrograde products and the development of new crystal regions [41,58]. Although fat is known to promote the production of amylose–lipid complexes, XRD (2θ: 13°) analysis did not detect their presence in this study, which was an unexpected result [23]. Therefore, the chemical compositions of purple rice flour were expected to decrease swelling power, solubility, digestibility, and other properties.

3.2. Swelling Capacity and Solubility

The swelling capacity and solubility values of native and HMT modified purple rice flour are listed in Figure 1. Swelling capacity and solubility values declined after receiving treatment with both oven (OHMT) and autoclave (AHMT), while this decline increased along with an increase in temperature. AHMT exhibited a significant effect on both traits when compared with OHMT. The utilization of HMT caused the polymer structures in the granules to become more crystalline in nature, leading to a reduction in the amount of water needed to initiate and break down the granules at 100 °C and 120 °C. The interaction between the polymer of starch in both the crystalline and non-crystalline regions, as well as the phosphate content [59], fat–amylose complex [60], and the binding strength between the molecules may contribute to a lower swelling capacity [61].

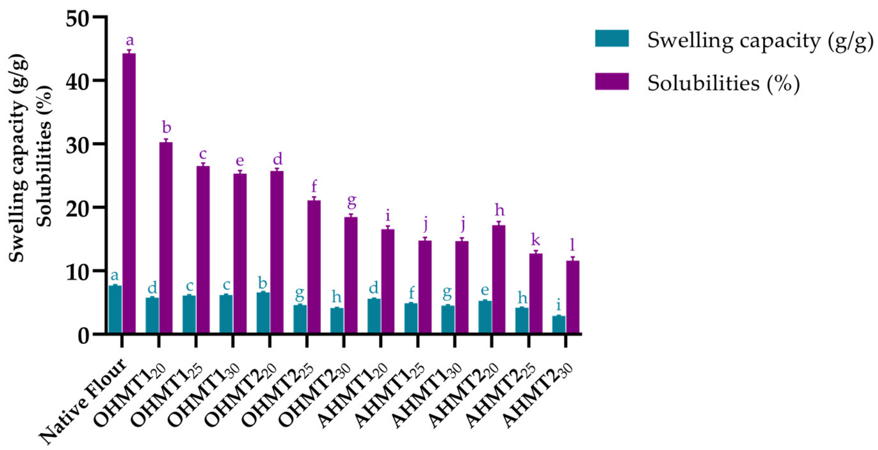

Figure 1. The swelling capacities and solubilities of native and HMT-treated purple rice flour samples. Means ± standard deviations from three replicate experiments. Different letters in the same column are considered significantly different according to Duncan's multiple comparison test ($p < 0.05$).

The HMT modification process is affected by factors such as energy and water content, which in turn can limit the mobility and interaction of various complexes, including amylose–lipid, amylose–amylopectin, and amylose–protein, leading to rearrangement and affecting the swelling power and solubility [9,20,40,41,62]. Figure 1 shows that increases in temperature, moisture content, and thermal sources can affect solubility. The swelling power of OHMT decreased by 3.55 (g/g) when compared to that of native flour, while AHMT swelling power was more dramatically decreased by 4.82 (g/g) than that which was recorded for native flour. The solubility of purple rice flour treated with OHMT and AHMT dropped to 25.78% and 32.65% when compared to that of native flour, respectively. A significant decrease in AHMT swelling power and solubility can be attributed to an increase in starch crystallinity [20]. HMT could have caused a decrease in swelling power, as a result of the rise in interactions between amylose–amylose and amylose–amylopectin [58]. The HMT treatment can reduce the availability of hydroxyl group (-OH) for hydration, leading to decreased swelling power and solubility [40]. The physicochemical properties of purple rice flour were significantly affected by its chemical components, and protein denaturation may prevent interaction with water, resulting in a decrease in both swelling power and solubility [15,41]. HMT has been observed to decrease both swelling capacity and solubility in sorghum [59,63], proso millet starch [63], corn [64], purple yam [20], and oats [65]. However, there was an increase in solubility, temperature, and moisture content in red sorghum starch [66]. This may have occurred as a consequence of the imperfect rearrangements of HMT, which may improve solubility by allowing starch molecules to disperse more easily in water [67,68].

3.3. The Impact of Three Cycles of HMT on Starch Digestibility

HMT and annealing are two common thermal treatments that are used to increase RS content. Starch is classified as either RDS, SDS, and RS based on digestibility. Table 2 shows the RS contents of native and HMT modified flours. The thermal treatment caused a decrease in RDS but an increase in SDS and RS. As compared to the native flour, the HMT-modified purple rice flour exhibited a proportional increase in both RS and SDS, based on the temperature and moisture content. RDS and SDS refer to the fractions that are converted to glucose within 20 and 120 min, respectively, while RS is the starch that is not hydrolyzed. The RDS, SDS, and RS contents of the OHMT modified purple rice flour were 45.49–52.82%, 26.64–36.38%, and 14.90–23.00%, respectively, while the AHMT contents were 38.22–48.40%, 32.50–42.69%, and 14.95–24.97%. Both treatments reduced the RDS by 5.95–13.28% and 10.37–20.55%, respectively. When compared to the native purple rice flour (NF), the SDS and RS contents of the OHMT modified purple rice flour increased to 2.22–9.74% and 2.74–10.84%, respectively, while the SDS and RS values of AHMT were 3.43–13.55% and 2.97–12.81%, respectively. Chung et al. [38] found that modifying corn, pea, and lentil starches using HMT resulted in a reduction in RDS and an increase in both SDS and RS. Similarly, Ahn et al. [12], Xiao et al. [17], and Cahyana et al. [9] observed an increase in SDS in sweet potato flour, tartary buckwheat flour, and green banana flour, respectively. Niba [69] also reported higher SDS levels in starch derived from various sources, including maize, cocoyam, plantain, potato, and rice. HMT treatment led to an increase in the RS content in both pearl millet starch and potato starch [70,71].

Table 2. RDS, SDS, and RS contents in OHMT and AHMT purple rice flour samples.

Samples	RDS (%)	SDS (%)	RS (%)
NF	58.77 ± 0.13 [a]	29.07 ± 0.19 [h]	12.16 ± 0.32 [i]
$OHMT1_{20}$	52.82 ± 0.30 [b]	26.64 ± 0.60 [i]	20.54 ± 0.30 [d]
$OHMT1_{25}$	51.01 ± 0.45 [c]	31.29 ± 0.21 [g]	17.70 ± 0.24 [f]
$OHMT1_{30}$	48.73 ± 0.15 [d]	36.38 ± 0.55 [d]	14.90 ± 0.39 [h]
$OHMT2_{20}$	48.23 ± 0.43 [d]	28.77 ± 0.18 [h]	23.00 ± 0.25 [b]
$OHMT2_{25}$	46.95 ± 0.24 [e]	31.77 ± 0.52 [fg]	21.28 ± 0.28 [cd]
$OHMT2_{30}$	45.49 ± 0.25 [f]	34.16 ± 0.50 [e]	20.35 ± 0.25 [d]
$AHMT1_{20}$	48.40 ± 0.42 [d]	32.50 ± 0.88 [f]	19.10 ± 0.46 [e]
$AHMT1_{25}$	45.00 ± 0.21 [f]	38.39 ± 0.60 [c]	16.60± 0.39 [g]
$AHMT1_{30}$	44.10 ± 0.32 [g]	40.96 ± 0.51 [b]	14.95 ± 0.18 [h]
$AHMT2_{20}$	38.22 ± 0.27 [j]	36.81 ± 0.14 [d]	24.97 ± 0.41 [a]
$AHMT2_{25}$	39.45 ± 0.28 [i]	39.06 ± 0.44 [c]	21.50 ± 0.16 [c]
$AHMT2_{30}$	40.23 ± 0.43 [h]	42.62 ± 0.59 [a]	17.16 ± 1.02 [fg]

The presented data are expressed as means ± standard deviations, and statistically significant differences between values in the same column are denoted by different letters ($p < 0.05$).

RS is a product of starch polymer structure rearrangement that cannot be degraded by amylase activity [10,72], while SDS consists of a very rigid amorphous region derived from retrograded products with its crystalline region partially destroyed during cooling. Starch crystallinity and digestion are primarily affected by moisture content, temperature, heating time, and the source of the starch. Higher levels of heat and humidity increase the mobility of interactions between polymer chains, resulting in a crystalline array of amorphous regions [73].

Thermal treatment of starch can lead to the production of resistant starch type III (RS3) [74], which is formed naturally through retrogradation when short-chain amylose molecules reassociate via hydrogen bonding after cooling from the autoclave process. In this study, the cooling process between HMT cycles was conducted before equilibrating the sample at −4 °C for 24 h, which can impact the development of small crystalline regions within starch granules [33]. The development of these new crystal areas can increase both crystallinity [75] as well as the contents of RS and SDS [76]. The ability to break down retrograded starch is influenced by the duration and temperature of storage [76]. One of the benefits of RS3 in comparison to other types of RS is that it can withstand heat more effectively, which means it remains stable during food processing [9]. Furthermore, RS3 can be utilized by probiotic bacteria, resulting in the production of short-chain fatty acids (SCFA) [77], it can act as a source of prebiotics that regulates the growth of probiotic bacteria, inhibits pathogens in the intestines, stimulates the immune system, and reduces constipation [78].

3.4. X-ray Diffraction Pattern

The X-ray diffraction patterns of the native and modified purple rice flour samples after oven (OHMT) and autoclave (AHMT) treatments are shown in Figure 2. A diffraction peak was observed in the native purple rice flour at 2θ = 15°, 17°, 18°, and 23°. A strong peak was observed at 15°, 17°, 18°, and 23° in the HMT samples. Additionally, an A-type starch diffraction pattern was observed that was not altered by the HMT flour on twelve modified samples. A-type contains distinct peaks at 15° and 23°, as well as a double peak at 17° and 18° [79]. As a result, although the intensity values of the peaks obtained from the HMT-modified purple rice flour were varied, significant changes in the types of X-ray diffractions were not detected. In their research, Kaur and Singh [80] studied different oat cultivars and found that the thermal treatment did not alter the X-ray diffraction (XRD) patterns of the starch samples, but resulted in increased intensity of peaks at 15° and 17.3°, which was attributed to the formation of new crystals or a change in the extent or number of crystalline areas. However, the peaks at 15° and 17.3° became more intense than the native starch, possibly due to the formation of new crystals or changes in the extent or number of crystalline areas. The same effect was observed in buckwheat starch [81], which also had an A-type crystalline structure with peaks at theta angle (2θ) around 15.24°, 17.14°, 18.04°, and 22.98°, the pattern of the diffraction peaks in buckwheat starch was not affected by alterations with HMT, but the intensity of the peaks was increased.

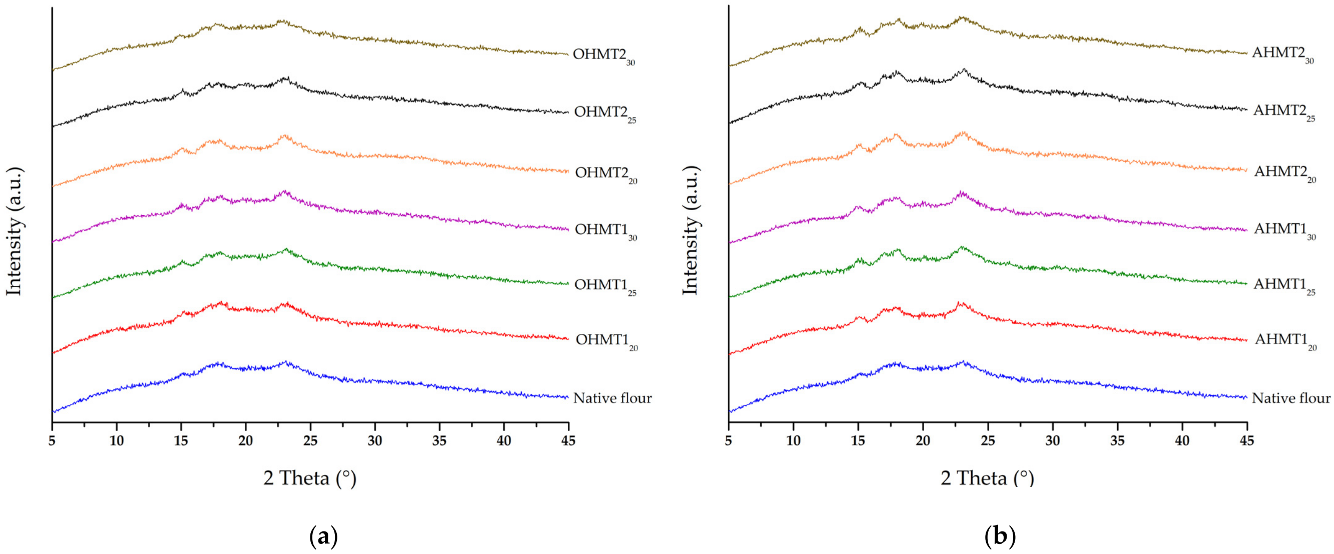

Figure 2. The native and HMT purple rice flour were analyzed using X-ray diffraction patterns after two treatments: (**a**) oven heat–moisture treatment (OHMT) and (**b**) autoclave heat–moisture treatment (AHMT).

HMT can also alter the intensity and crystallinity of purple rice flour. Table 3 summarizes the peak intensity and crystallinity values of both native and treated purple rice flour. The peak intensities of all samples increased when compared to the native purple rice flour. This has also been mentioned in conjunction with cassava flour [82], purple yam flour [20], wheat starch [83], bean starch [68], arrowroot [62], corn starch [84], and waxy maize starch [8]. During the HMT process, moisture content and thermal energy were generated and caused a structural rearrangement in starch granule crystals and the displacement of double helices, which resulted in an improvement in crystal formation [73]. In addition, most forms of cereal starch, including rice starch, have an A-type crystalline structure for which low amylose has been found to be more effective at crystallinity than high-amylose starch because the molecules of amylopectin can form a double helix that is required for the crystal structure. The HMT of starch was processed at a temperature of about 120 °C to increase crystallinity and ultimately damage the starch. Applying high temperatures caused the covalent bonds to break, which in turn caused the double helix structure of amylopectin to unwind. This created a new arrangement with a higher density, resulting in the formation of starch granule crystals [85].

Table 3. The peak intensities and relative crystallinity values of native and HMT purple rice flour samples.

Samples	Peak Intensities 2θ (°)				Relative Crystallinity (%)
	15	17	18	23	
NF	1366	1606	1664	1662	21.09 [k]
$OHMT1_{20}$	1496	1685	1710	1784	30.20 [f]
$OHMT1_{25}$	1442	1625	1685	1747	25.21 [j]
$OHMT1_{30}$	1465	1657	1701	1765	27.25 [h]
$OHMT2_{20}$	1565	1754	1785	1802	35.21 [b]
$OHMT2_{25}$	1527	1721	1732	1785	32.19 [d]
$OHMT2_{30}$	1501	1695	1708	1767	30.14 [f]
$AHMT1_{20}$	1513	1708	1725	1760	31.29 [e]
$AHMT1_{25}$	1485	1685	1702	1732	26.42 [i]
$AHMT1_{30}$	1468	1671	1681	1712	25.20 [j]
$AHMT2_{20}$	1582	1782	1784	1823	37.25 [a]
$AHMT2_{25}$	1535	1761	1745	1796	33.70 [c]
$AHMT2_{30}$	1507	1711	1715	1741	28.30 [g]

The presented data are expressed as means ± standard deviations, and statistically significant differences between values in the same column are denoted by different letters ($p < 0.05$).

All of the types of flour that were changed by treatment with an oven or an autoclave revealed a higher relative crystallinity value than that of the native purple rice. $OHMT2_{20}$ and $AHMT2_{20}$ revealed high crystallinity values of 35.21% and 37.25%, respectively. Despite AHMT samples generally exhibiting higher relative crystallinity values than OHMT samples, some AHMT samples, specifically $AHMT1_{30}$ and $AHMT2_{30}$, displayed lower crystallinity values than their $OHMT1_{30}$ and $OHMT2_{30}$ counterparts. The pressure in an autoclave is an important factor that can affect the modification of starch and its resulting crystallinity [86]. When starch is subjected to high pressure, the starch molecules are forced to pack more tightly together, resulting in increased crystallinity. The pressure also helps to penetrate the starch granules and allows for more efficient modification [87]. AHMT involves the use of high pressure and steam to heat, while OHMT uses dry heat to modify the purple rice flour. However, excessive moisture content in purple rice flour can also have a negative effect on the modification process. If the moisture content is too high, the starch granules may become too swollen and burst, leading to a loss of starch structure and a decrease in crystallinity [88]. This explains why some AHMT samples at 25% and 30% moisture content had lower crystallinity values despite the overall trend of higher values. Therefore, optimizing the parameters of AHMT, such as moisture content, is crucial to achieving the desired crystallinity values for modified starch granules. Additionally, the use of a three-cycle treatment method in AHMT, which applies significant pressure, can improve the connection among starch molecules and enhance crystalline perfection or create new crystals in the crystalline area, further improving the properties of modified starch granules [40,89]. This study employed ovens and autoclaves in the HMT process, which had an impact on several characteristics of the flour, including swelling capacity, solubility, digestibility, and crystallinity. These differences in characteristics were related to the differentials in heat transfer strength observed between the two thermal sources [10,90].

The autoclave caused a greater amount of heat transfer compared to the oven, resulting in more even exposure across the entire sample [90]. This increased heat transfer may affect the movement of starch polymer chains, allowing for faster modification of starch through autoclaving [91]. Due to the greater heat transfer in the autoclave, it is possible for water molecules in the polymer structure to evaporate, causing it to reorganize into a more crystalline structure. This will ultimately impact the swelling power, solubility, and digestibility of the sample [9,13,20,92–95]. Various studies have indicated that altering the physical properties of starch or flour through methods such as using ovens, autoclaves, osmotic pressure, or microwaving can lead to diverse physicochemical characteristics, even when the temperature and moisture content are theoretically constant. Additionally, the homogeneity of heat transmission across the granules can be improved by maintaining a consistent flour particle size and restricting the number of samples [95–99].

3.5. Bioactive Compound and Antioxidant Activities

As can be seen in Figure 3, the total phenolic content (TPC), and total anthocyanin content (TAC) of modified purple rice flour were found to be significantly lower than that of the native purple rice flour. TPC and TAC values of the OHMT modified purple rice flour were 3.37–3.70 mg GAE/g and 163.20–295.27 mg/100 g, meanwhile AHMT contents were 4.19–4.45 mg GAE/g and 310.17–340.37 mg/100 g, respectively. In both treatments, the reduction in TPC was within the range of 53.16 to 57.34% for OHMT and 43.67 to 46.96% for AHMT, respectively. The TAC values of the OHMT and AHMT modified purple rice flour decreased within the ranges of 44.96 to 69.59% and 36.57 to 42.16%, respectively, when compared to the native purple rice. Accordingly, HMT may have caused damage to heat-sensitive phenolic components [100,101]. Walter et al. [102] reported a significant decrease in the total soluble TAC of rice with light brown, red, and black pericarps after being treated by thermal processing. This resulted in certain phenolic compounds leaching into the water. Anthocyanins are molecules that are highly susceptible to breakdown due to their high reactivity. Raising the temperature during cooking can cause the pyrylium rings of anthocyanins to open, which can lead to the separation of the glycoside linkage and the creation of a chalcone structure (a colorless form), indicating the first stage of anthocyanin breakdown. [103]. Most authors have observed a reduction in the quantity of anthocyanins after applying different heat treatments [101,104,105].

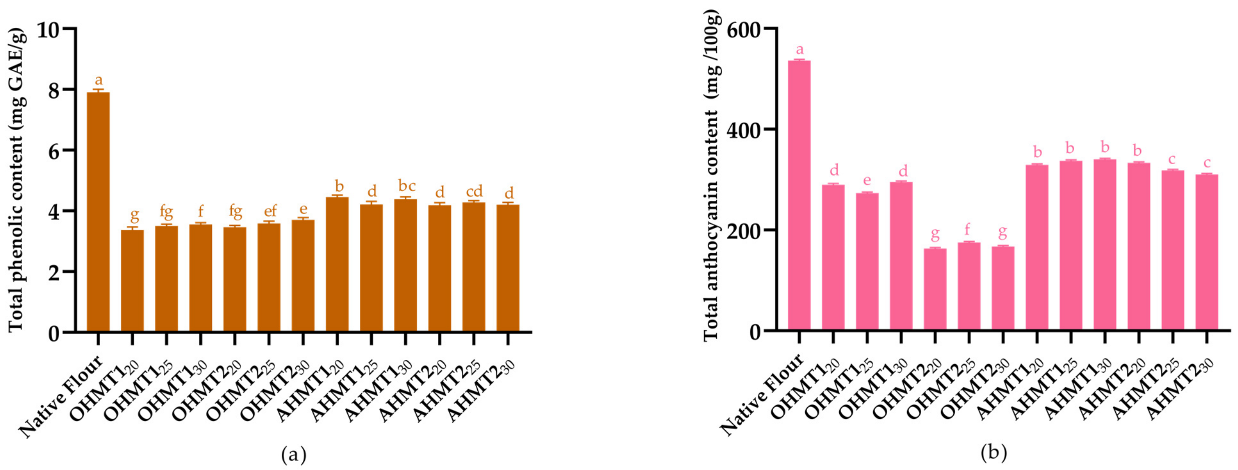

Figure 3. Bioactive compound of native and HMT-treated purple rice flour samples. (**a**): total phenolic content, (**b**): total anthocyanin content. Means ± standard deviations from three replicate experiments. Different letters are considered significantly different according to Duncan's multiple comparison test ($p < 0.05$).

The DPPH and ABTS radical scavenging activity of HMT were remarkably degraded relative to those of the native purple rice flour ($p < 0.05$) (Table 4). The DPPH and ABTS values of OHMT-modified flour were 1.97–3.06 and 0.87–2.07 mg TE/g, respectively; meanwhile, AHMT contents were found to be 3.23–3.71 and 2.18–2.60 mg TE/g, respectively. In both treatments, the reduction in DPPH ranged from 73.20 to 82.75% for OHMT and from 67.51 to 71.72% for AHMT, while the ABTS values were within the range of 85.59 to 93.95% for OHMT and 81.91 to 84.83% for AHMT, respectively. The results indicated that thermal processing can cause degradation of phenolic compounds, anthocyanins, and antioxidant activity. This degradation is attributed to the breakdown of these compounds into other products and the vaporization that occurs during the heating process [106–108]. The authors hypothesize that these changes that occurred in the contents of individual bioactive compounds in native purple rice flour affected the antioxidant properties of the substances including the DPPH and ABTS radical scavenging activities. It is reasonable to assume that the variations in the contents of individual phenolic acids in native purple rice flour occurred due to the cultivar and HMT conditions that affected their antioxidant properties, particularly their ABTS radical scavenging activity. The results from the DPPH and ABTS assays indicated that the antioxidant capacity of the purple rice flour and modified flour appears to be related to the phenolic and anthocyanin content. Furthermore, it was found that AHMT was associated with higher antioxidant activity when compared to OHMT.

Table 4. Antioxidant activities of native and HMT-treated purple rice flour samples.

Samples	**Antioxidant Activities**	
	DPPH Radical Scavenging Activity (mg TE/g)	**ABTS Radical Scavenging Activity (mg TE/g)**
NF	11.42 ± 0.11 [a]	14.37 ± 0.07 [a]
$OHMT1_{20}$	2.59 ± 0.06 [g]	2.07 ± 0.06 [d]
$OHMT1_{25}$	2.77 ± 0.07 [f]	1.84 ± 0.04 [e]
$OHMT1_{30}$	3.06 ± 0.06 [e]	1.92 ± 0.05 [e]
$OHMT2_{20}$	2.47 ± 0.05 [g]	1.69 ± 0.05 [f]
$OHMT2_{25}$	2.29 ± 0.05 [h]	1.39 ± 0.05 [g]
$OHMT2_{30}$	1.97 ± 0.07 [i]	0.87 ± 0.04 [h]
$AHMT1_{20}$	3.61 ± 0.05 [b]	2.60 ± 0.05 [b]
$AHMT1_{25}$	3.71 ± 0.04 [b]	2.42 ± 0.06 [c]
$AHMT1_{30}$	3.64 ± 0.04 [b]	2.47 ± 0.08 [bc]
$AHMT2_{20}$	3.64 ± 0.04 [b]	2.20 ± 0.08 [d]
$AHMT2_{25}$	3.37 ± 0.07 [c]	2.34 ± 0.04 [c]
$AHMT2_{30}$	3.23 ± 0.03 [c]	2.18 ± 0.06 [d]

The presented data are expressed as means ± standard deviations, and statistically significant differences between values in the same column are denoted by different letters ($p < 0.05$). TE: Trolox equivalent, DPPH: 2,2-diphenyl-2-picrylhydrazyl radical scavenging activity, ABTS: [2, 2-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt] radical cation scavenging activity.

AHMT offers the benefit of requiring a shorter processing time while maintaining higher levels of TPC, TAC, and antioxidant activity than OHMT, which is ideal for producing health food using purple rice flour. Purple rice flour processed with AHMT can potentially have higher amounts of RS and better properties of bioactive substances compared to other flours. However, further research is necessary to investigate the effects of OHMT and AHMT on the breakdown mechanisms of phenolic acids and anthocyanins. Our findings suggest that both OHMT and AHMT can result in a reduction in TPC and TAC values, which subsequently reduces relevant antioxidant activity (as indicated by DPPH and ABTS assays). These observations confirm that OHMT and AHMT processes are significant factors that impact the degradation of bioactive compounds and antioxidant activity.

3.6. Correlation of TPC, TAC, DPPH, and ABTS

The Pearson's correlation analysis (Table 5) demonstrated a substantial positive correlation between TPC and TAC, suggesting that anthocyanins were the primary phenolic compounds present in the colored rice grains. This result is consistent with the findings of a prior investigation [101,107]. Furthermore, specific bioactive substances (TPC and TAC) were highly correlated with DPPH and ABTS antioxidant activity. The correlation analysis showed that the antioxidant potential of the sample was mainly attributed to the phenolic compounds, which may function primarily as reducing agents instead of radical scavengers, as evidenced by the slightly stronger positive correlation of DPPH value with the TPC and TAC values.

Table 5. The correlation between total phenolic content (TPC), total anthocyanin content (TAC), DPPH radical scavenging activity, and ABTS radical scavenging activity was determined using Pearson's coefficient for all samples of purple rice flour.

	TPC	TAC	DPPH	ABTS
TPC	1.000	0.849 *	0.979 *	0.964 *
TAC		1.000	0.858 *	0.813 *
DPPH			1.000	0.993 *
ABTS				1.000

* Correlations were considered significant at $p < 0.01$ (two-tailed).

3.7. Effects HMT on Morphology

A scanning electron microscope was used to assess the morphology of the starch granules (Figure 4). The micrographs of purple rice flour revealed the existence of polyhedral granules. In all treatments, HMT did not have an impact on the purple rice flour granule morphology, but it did modify the surface of the particles, resulting in a rough and pitted appearance compared to the native flour.

Figure 4. Scanning electron microscope images of purple rice flour exposed to different treatments. (**A**): native flour, (**B**): oven heat–moisture treatment (OHMT2_{20}), (**C**) AHMT: autoclaved heat–moisture treatment (AHMT2_{20}).

Several studies have suggested that the size and shape of starch granules in plants such as taro, maize, cassava, wheat, rice, and potato remain unaffected by HMT treatment [58,109,110]. According to Kawabata et al. [111], HMT treatment led to the formation of cracks on the surface of maize and potato starch granules, as well as a certain level of granule hollowing. A study by Zavareze et al. [112] observed that starch treated with high moisture concentrations of 20% and 25% displayed disintegration, in contrast to the native starch samples.

4. Conclusions

The modified purple rice flour by heat–moisture treatment (OHMT and AHMT) for RS is a novel blend with new food with antioxidant properties. Our study examined the effects of OHMT and AHMT methods on modifying the physical, chemical, and digestibility characteristics of purple rice flour in the north of Thailand. The results of our study showed that both methods increased the SDS and RS content and raised the X-ray diffraction intensity. However, AHMT had a greater impact on all physicochemical and digestibility variables compared to OHMT. The relative crystallinity of the samples increased, ranging from 4 to 14% for OHMT and 4–16% for AHMT. This increase in the relative crystallinity indicates an improvement in the resistance to enzymatic digestion and thus a slower glucose release, making it beneficial for people with diabetes.

Furthermore, our study showed that both OHMT and AHMT can preserve important compounds such as phenolic compounds, anthocyanins, and antioxidant activities in purple rice flour. The treated flour could be used as a source of RS to produce healthy foods, such as pasta, bread, biscuits, and crackers. The incorporation of purple rice flour into these foods could add a unique purple color and improve the antioxidant properties. Our study highlights the potential of using modified purple rice flour as a functional ingredient in the food industry.

The AHMT method showed the greatest impact on improving the physical, chemical, and digestibility characteristics of the purple rice flour. The modified flour can be incorporated into various foods to produce healthier and more nutritious products. The use of modified purple rice flour in the food industry offers great potential for creating functional foods that provide health benefits beyond basic nutrition. With its unique properties, purple rice flour could help food manufacturers to differentiate their products and meet the growing demand for healthy and natural ingredients. Overall, our study suggests that the modified purple rice flour has significant potential for application in the food industry and further research is needed to explore its potential uses.

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