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Comparative analysis of the physicochemical properties and antioxidant activities of organic Riceberry rice from different growing locations

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Abstract The study revealed significant variations in the physicochemical and antioxidant properties of organic Riceberry rice cultivated in four districts of Kalasin, Thailand. Rice from Site 4 (Kham Muang) showed the highest antioxidant activities, with elevated values in DPPH radical scavenging (923.17 µg Trolox/g), FRAP (1001.39 µg $FeSO_4$/g), total phenolic content (816.26 µg GAE/g), and total anthocyanin content (51.86 g c3g/100g), indicating favorable conditions for phenolic compound biosynthesis. Conversely, rice from Site 3 (Khong Chai) had the highest amylose content (14.20%), resulting in lower peak viscosity and greater setback viscosity, which suggests increased starch retrogradation and firmer rice texture. Site 1 (Mueang) rice displayed the highest onset and peak gelatinization temperatures, indicating a more stable starch structure, while Site 4 had the highest peak viscosity (189.58 RVU) and breakdown, reflecting a starch with greater water absorption and gel-forming capacity. These variations are linked to differentiate in soil texture, drainage, and environmental factors, which affected the rice's nutrient uptake, antioxidant activity, and starch properties.

Keywords: Riceberry rice, Antioxidant activity, Physicochemical properties, Thermal properties, Environmental influence

Introduction

Riceberry rice a special kind of purple-black rite originated from Thailand has gained fame due to its nutritional richness and high antioxidant level. This variety is preferred for its high concentrations of bioactive compounds including phenolic acids, flavonoids, and anthocyanin which explain its antioxidant activities as well as potential health benefits (Butsat and Siriamornpun, 2010). Nevertheless, the quality attributes of Riceberry rice such as its physicochemical properties, antioxidant activities as well as thermal properties would differ depending on the environment and growth sites.

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Organic farming therefore presents a new method of farming that differs from the conventional type of farming which more often uses chemicals to control pests and diseases promising a solution to the growing issue of food insecurity. Organic fertilization improves the biological, chemical, and physical qualities of the soil resulting in improved growth and yield of plants since it has a high content of organic compositions and microbial activity (Oyedeji *et al*., 2014; Mitran *et al*., 2018). Also, the combined effects of genetic and environmental factors; the nutrients in the soil, temperature, and moisture availability are key factors affecting the quality of rice grains (Xu *et al*. 2015). These because nutrient availability and moisture are elements that must be managed in order to achieve the best result from rice production and quality.

Phenolic compounds, flavonoids, and anthocyanins are inversely related to the free radical scavenging ability or antioxidant activity of rice (Sharma *et al*., 2014). These bioactive compounds are produced as a result of metabolic reaction sequences which are environmental stimuli sensitive. For example, Peanparkdee *et al*. (2020) have established that light, temperature, and nutrient contents of the soil play critical roles in determining the levels of phenolics and flavonoids in the rice grains and their antioxidant potential.

Starch properties such as gelatinization and retrogradation of rice starch are some of the quality factors of rice especially in food processing and product formulation. These thermal properties depend upon amylose and amylopectin which in their content differ with growth conditions. Depending on the conditions under which it is derived, starch has the potential to undergo several changes that may have an impact on its thermal stability and gelatinization characteristics: soil moisture, temperature, and nutrient content (Zhang *et al*., 2018; Compart *et al*., 2023).

The present research compared the physicochemical characteristics, antioxidants as well as thermal stability of the Riceberry rice grown under organic farming at four locations in Kalasin, Thailand. Therefore, in light of these properties, the study was assessed environmental effects on the quality attributes of the Riceberry rice and would become information for improving the cultivation practices in order to improve the nutritional and functional values of the rice.

Materials and methods

Sample preparation

Organic Riceberry rice samples were sourced from four different locations in Kalasin province, northeastern region of Thailand including Mueang, Yang

Talat, Khong Chai, and Kham Muang districts as enlisted in Table 1. The harvested grains were threshed and de-husked to get the brown rice which were further ground and passed through 100-mesh screens to achieve a fine particle size. For the analysis of moisture content, the processed rice samples were dried at 110 °C until the samples reached a constant weight. All procedures were replicated three times for the accuracy of data, while the results were presented on a dry matter basis. Samples were stored at −20 °C until further analysis.

Table 1. Description of the sites where the organic Riceberry rice samples were obtained in this study

Site	Source	Soil properties[a]
1	Mueang district	This group of soils is poorly drained, and coarse-textured that occurs on low-lying terrain. They have very low fertility.
2	Yang Talat district	This group of soils is well-drained, deep, and coarse-textured and develops from alluvial deposits or wash materials on the uplands of alluvial terraces, fans, or erosional surfaces in areas of low precipitation. They have low fertility. Soil reaction is a strong acid.
3	Khong Chai district	This group of soils is poorly drained or somewhat poorly drained, fine textured (clay loam or silty clay loam) to clay or silty clay, that commonly occurs on flood plains and low-lying terraces or alluvial fans. They are moderate fertility with reactions ranging from medium acid to neutral.
4	Kham Muang district	Very deep grey loamy soils from alluvial deposit, poorly drained to somewhat poorly drained, medium textured (silt loam grading to silty clay loam) soils that developed mostly in the areas of alluvial plain or flood plain.

[a] Cited from Land Development Station (2015).

Chemicals and reagents

All chemical reagents, including 1,1-diphenyl-2-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-tripyridyl-s-triazine (TPTZ), Folin-Ciocalteu reagent, and standards like gallic acid and quercetin, along with amylose from potatoes, were obtained from Sigma-Aldrich, St. Louis, MO, USA. All used reagents were analysed the grade to ensure accuracy and reliability in the results.

Proximate composition

The proximate composition of the rice samples, including moisture, protein, fat, carbohydrate, ash, and fiber, was determined following the methods outlined by the Association of Analytical Chemists (AOAC, 2010). The protein content was calculated using a conversion factor of 5.95, suitable for rice flour. Carbohydrate content, reported as nitrogen-free extract, was calculated by subtracting the combined weight of moisture, protein, fat, ash, and fiber from the total dry matter (Adeduntan, 2005).

Determination of amylose content

Amylose content in rice flour was estimated according to the method developed by the American Association of Cereal Chemists (method 76–13, 1999). For this procedure, rice flour weighing 100 mg was subjected to the following reagents; 1 mL of 95% ethyl alcohol and 9 mL of 1N sodium hydroxide (NaOH). The mixture was allowed to rest at RT for 10 min and then the mixture was heated in a water bath at 100 °C for 10 minutes. The mixture was cooled to room temperature for at least 2 h before the final volume of the sample was adjusted to 100 mL with distilled water and mixed using a vortex mixer.

For analysis, 5 mL of the prepared solution was diluted with 50 mL of distilled water. Then, 2 mL of 1N acetic acid and 2 mL of iodine solution, which contains 0.2 g of iodine and 2.0 g of potassium iodide dissolved in 100 mL of distilled water, were added. The final volume was adjusted to 100 mL with distilled water. Subsequently, the absorbance of reactants was recorded at 620 nm after a resting period of 20 min. The percentage of amylose content in the extracts was determined by making use of a calibration curve of amylose concentrations in distilled water from potato starch.

Assessment of antioxidant activity

Sample extraction

To extract the samples, 1 gram of rice flour was mixed with 3 mL methanol in the 15 mL capacity test tubes and was vortexed for 30 sec then sonicated at room temperature for 20 minutes. In the process of sonication, the samples were vortexed two times. The mixture was then centrifuged at 10,000 rpm for 10 min this would help in separating the rice flour debris. The supernatant was then transferred to a 10 mL flask. The pellet was re-extracted with 3 mL methanol, and its supernatants were combined with that of the previous supernatant and then diluted to 10 mL with methanol. The extracts were kept at −20 °C until the use of the samples for analysis.

DPPH radical scavenging activity

The DPPH radical scavenging activity was assessed by a slightly modified method by Wanyo *et al*. (2016). A 0.20 mL sample extract was then aliquoted with 2 mL of 0.1 mM DPPH in methanol, vortexed for 1 min, and placed in the dark at room temperature for 30 min to enable the reduction to happen. The absorbance was measured with the help of a spectrophotometer at the wavelength of 517 nm. A calibration curve with different concentrations of Trolox (50-500 µM) was also established for the determination of DPPH radical scavenging activity which was given as µmol Trolox equivalent/g of the sample.

Ferric reducing/antioxidant power (FRAP) assay

The FRAP assay was done using a slightly modified method from Wanyo *et al*. (2016). FRAP reagent was made by mixing 100 mL of 0.3 M Acetate buffer with a pH of 3.6, 10 mL of 10 mM TPTZ in 40 mL of hydrochloric acid, 10 mL of 20 mM $FeCl_3 \cdot 6H_2O$ in distilled water and completing it up to 120 mL with the same distilled water with the ratio 10:1:1. It was therefore warmed to 37 °C. For the assay, 8 mL of FRAP reagent, 180 µL of Milli-Q water, and 60 µL of the sample, standard or blank were mixed in test tubes, and incubated in a water bath set at 37 °C for 4 min, then, the absorbance was measured at 593 nm. Antioxidant activities were quantified using a standard curve from $FeSO_4 \cdot 7H_2O$ standards.

Quantification of phenolic compounds

Determination of total phenolic content

Total phenolic content (TPC) was determined using the Folin–Ciocalteu reagent as per the method of Wanyo *et al*. (2016). Briefly, 300 µL of the extract was aliquoted with 2.25 mL of Folin–Ciocalteu reagent, the solution was diluted tenfold with distilled water. The mixture was stirred and then allowed to attain room temperature for 5 min. Then, 2.25 mL of a sodium carbonate solution (60 g/L) was added, and the mixture was incubated at an ambient temperature for 90 min. The absorbance was measured at 725 nm, and TPC was expressed as micrograms of gallic acid equivalents per gram of dried sample (µg GAE/g).

Determination of total flavonoid content

Determination of the total flavonoid content (TFC) was done using the colorimetric method described by Wanyo *et al*. (2016) with some modifications. A 0.5 mL sample extract was mixed with 2.25 mL of distilled water in a test tube. Then, 0.15 mL of Sodium nitrite solution (5%) was added and allowed to stand for 6 min followed by the aliquot added with 0.3 mL of aluminum chloride hexahydrate solution (10%) with a 5 min waiting time. Next, 1.0 mL of 1M sodium hydroxide was added, and the mixture was vortexed thoroughly. The absorbance was measured at 510 nm, and TFC was expressed in micrograms of quercetin equivalent per gram of dried samples (µg QE/g).

Determination of total anthocyanin content

Total anthocyanin content (TAC) was analyzed using a spectrophotometric pH-differential method according to the method of Lee *et al*. (2005). One gram of Riceberry rice flour was extracted with distilled water at a 1:10 (w/v) ratio under static conditions at room temperature. After extraction, the mixture was centrifuged at 10,000 rpm and 4 °C for 20 min. The absorbance was determined by the formula $A = (A\lambda510 – A\lambda700)$ at pH 1.0 $– (A\lambda510 – A\lambda700)$ at pH 4.5. Total monomeric anthocyanine content in petals was estimated and presented as cyanidin-3-glucoside equivalents.

Determination of pasting properties

The rheological properties of rice flour pastes were determined using a Rapid Visco Analyzer (RVA; Super-4, Newport Scientific Pty Ltd., Australia). To perform the analyses, 3.0 g of rice flour on a dry weight basis was dispersed with 25 mL of distilled water in an RVA canister. The temperature program used was as follows: The sample was heated to a temperature from 50 °C to 95 °C at the rate of 12 °C per min and the temperature was maintained at 95 °C for 5 min, The sample was then cooled to 50 °C at the same rate and the temperature maintained at 50 °C for 2 min. The RVA offered pasting temperature, peak viscosity, breakdown, final viscosity, and setback values which are relevant in reflecting the characteristics of rice flour during cooking and processing.

Determination of thermal properties

Characterization of thermal properties of rice flour samples was measured using a Differential Scanning Calorimeter (DSC; Q200, TA Instruments, Newcastle, DE, USA). In this study, 40 mg of rice flour on a dry basis was Ali-batched and transferred into an aluminum sample pan and heated from 40 °C to 160 °C at a rate of 5 °C per min. The parameters recorded were onset temperature (To), peak temperature (Tp), conclusion temperature (Tc), and enthalpy of gelatinization (ΔH). These measurements reveal information concerning the thermal properties and gelatinization behavior of rice starch.

Statistical analyses

Data were analyzed using analysis of variance (ANOVA) in a completely randomized design, with Duncan’s Multiple Range Test used for post-hoc comparisons. Each determination was performed in triplicate to ensure reliability, and results were expressed as mean ± standard deviation. Differences were considered statistically significant at $p < 0.05$.

Results

Site characteristics and soil properties

The samples of organic Riceberry rice were collected from four different locations, and each location had different types of soil properties detailed in Table 1. Site 1 is the Mueang district, which consists of a portion of coarse-textured, poorly drained soil that is nearly wholly unproductive due to its low fertility. This contrasts with the Yang Talat district (Site 2), which found to be well-drained, coarse-textured, poor fertility, and strongly acidic soils that are formed from alluvial deposits. Fine-textured, somewhat to moderately alkaline, poorly to somewhat poorly drained, and moderately rich soils are the defining characteristics of the Khong Chai district (Site 3). The medium-textured, alluvial loam in the Kham Muang district (Site 4) is extremely rocky and poorly drained.

Chemical compositions

The chemical composition of the Riceberry rice from the four different sites, there are variations of lipid, protein, crude fiber, and ash content as well as amylose content while there were no significant differences in the moisture and carbohydrate contents (Table 2). Moisture content ranged from 8.98% to 9.28% across all sites ($p > 0.05$), indicating that environmental conditions or post-harvest handling practices were similar for all sites. Lipid content also differed, as was found in Site 3 which had the highest estimate at 3.29% while the lowest estimate was recorded to be 2.99% in Site 4. Protein content ranged from 7.48% to 8.14%, the highest amount was observed at Site 4 and the lowest quantity was observed at Site 3. The crude fiber content recorded a high variability across the sites with Sites 1 and 4 recording the highest amounts at 1.30% and 1.27%, respectively. Ash contents which represent the total minerals content in the samples showed a high fluctuation in the sites, with the highest content in Site 4 (1.56%) and the slowest content in Site 2 (1.43%). For the carbohydrate content, there were no significant differences at all in the four sites ($p > 0.05$). This indicates that carbohydrate accumulation in Riceberry rice is likely not strongly affected by the environmental or soil conditions in these regions. The amylose content which ranged from 12.46% at Site 1 to 14.20% at Site 3 was different ($p < 0.05$).

Table 2. Chemical compositions of organic Riceberry rice from four sites

Compositions (%)	Sample Sites			
	1	2	3	4
Moisture content ^{NS}	8.98±0.35	9.28±0.37	9.15±0.31	9.27±0.37
Lipid content	3.25±0.18^{ab}	3.14±0.13^{ab}	3.29±0.11^{a}	2.99±0.08^{b}
Protein content	7.97±0.11^{ab}	7.49±0.11^{b}	7.48±0.06^{b}	8.14±0.07^{a}
Crude fiber	1.30±0.02^{a}	0.94±0.01^{c}	1.07±0.01^{b}	1.27±0.01^{a}
Ash content	1.52±0.01^{b}	1.43±0.01^{c}	1.45±0.01^{c}	1.56±0.01^{a}
Carbohydrate ^{NS}	76.98±0.67	77.72±0.63	77.56±0.50	76.77±0.54
Amylose content	12.46±0.09^{c}	13.23±0.13^{b}	14.20±0.10^{a}	13.96±0.08^{b}

Values are expressed as mean ± standard deviation (n = 3). Means with different letters in the same row were significantly different at the level $p < 0.05$ and NS = not significant.

Antioxidant activity

The antioxidant activities of Riceberry rice from the four sites were measured using various assays, including DPPH radical scavenging activity, ferric reducing antioxidant power (FRAP), total phenolic content (TPC), total flavonoid content (TFC), and total anthocyanin content (TAC), as shown in Table 3. The results of DPPH ranged from 601.02 µg Trolox/g at Site 1 to 940.63 µg Trolox/g at Site 3, with the highest values recorded at Sites 2, 3, and 4. These were not significantly different from each other but were significantly higher than Site 1. FRAP values also varied significantly, with the lowest activity at Site 1 (644.98 µg $FeSO_4$/g) and the highest at Sites 2 and 3 (1089.95 and 1090.24 µg $FeSO_4$/g, respectively). Site 4 showed a relatively high FRAP value (1001.39 µg $FeSO_4$/g), comparable to Sites 2 and 3.

Table 3. Antioxidant activity of organic Riceberry rice from four sites

Qualification	Sample Sites			
	1	2	3	4
DPPH (µg Trolox/ g)	601.02±26.39^{b}	935.44±46.23^{a}	940.63±54.06^{a}	923.17±52.41^{a}
FRAP (µg $FeSO_4$/g)	644.98±80.20^{b}	1089.95±89.45^{a}	1090.24±8.72^{a}	1001.39±91.05^{a}
TPC (µg GAE/g)	585.02±33.61^{b}	882.99±88.68^{a}	839.55±64.02^{a}	816.26±43.30^{a}
TFC (µg QE/g)	9.42±0.58^{ab}	9.92±0.89^{a}	8.25±0.94^{b}	9.36±0.69^{ab}
TAC (g c3g/100g)	18.39±0.37^{d}	41.06±0.80^{c}	49.09±0.93^{b}	51.86±0.97^{a}

Values are expressed as mean ± standard deviation (n = 3). Means with different letters in the same row were significantly different at the level $p < 0.05$.

The TPC and TFC were significantly higher at Sites 2 and 4, respectively. Phenolic compounds, which are secondary metabolites in plants, play a crucial role in protecting rice against environmental stressors and pathogens. The high TPC at Site 2 suggests that environmental conditions, such as soil nutrient levels and water availability, may have favored phenolic compound production. The

TAC values have also relatively fluctuated across the sites with the minimum value of 18.39 g c3g/100g in Site 1 and the maximum value of 51.86 g c3g/100g in Site 4.

Pasting properties

The pasting properties of rice from each cultivation site of Riceberry rice are presented in Table 4. The properties that are peak viscosity, trough viscosity, breakdown, final viscosity, setback, peak time, and pasting temperature can explain the cooking and processing of the rice. The highest peak viscosity or the maximum viscosity achieved at the highest temperature during the test was recorded to be 189.58 RVU for Site 4 and the lowest PV was 167.47 RVU for Site 3. Sites 1 and 2 had the mediate ratio value with the observation of 183.80 RVU and 177.38 RVU, respectively and the F test showed significantly different ($p < 0.05$) among all the sites. Trough viscosity following the peak viscosity was also highest at Site 4 (184.14 RVU) and least at Site 1 (144.14 RVU), while Sites 2 and 3 were moderate with 156.31 RVU and 144.64 RVU, respectively.

Breakdown viscosity, which expresses the stability of the starch granules under heat and shear, was the highest for Site 4 (32.44 RVU) and lowest at Site 3 (26.03 RVU), with Sites 1 and 2 showing intermediate values of 28.28 RVU and 26.87 RVU, respectively. The final viscosity that indicated the ability of the starch to form gel when cooled was the highest value at Site 4 (252.31 RVU) and the lowest at Site 1 (202.64 RVU), while Sites 2 and 3 indicated intermediate values of 232.94 RVU and 215.88 RVU, respectively.

Table 4. The pasting property of organic Riceberry rice from four sites

Parameters	Sample Sites			
	1	2	3	4
Peak Viscosity (RVU)	183.80±1.59^{b}	177.38±1.78^{c}	167.47±1.22^{d}	189.58±1.06^{a}
Trough Viscosity (RVU)	144.14±1.78^{c}	156.31±1.25^{b}	144.64±0.94^{c}	184.14±1.86^{a}
Breakdown (RVU)	28.28±0.70^{b}	26.87±0.42^{c}	26.03±0.97^{c}	32.44±0.34^{a}
Final Viscosity (RVU)	202.64±2.33^{d}	232.94±3.44^{b}	215.88±4.54^{c}	252.31±5.43^{a}
Setback (RVU)	47.59±1.89^{c}	55.56±1.21^{b}	62.86±3.17^{a}	58.22±2.39^{b}
Peak Time (mim)	6.10±0.04^{a}	5.93±0.05^{b}	5.90±0.06^{b}	6.05±0.06^{a}
Pasting Temperature (ºC) ^{NS}	79.32±0.38	79.48±0.58	78.40±0.35	78.40±0.21

Values are expressed as mean ± standard deviation (n = 3). Means with different letters in the same row were significantly different at the level $p < 0.05$ and NS = not significant.

The setback viscosity, which is the measure of the extent of starch retrogradation or crystallinity during the cooling period, was highest at Site 3 (62.86 RVU) and lowest at Site 1 (47.59 RVU), while Sites 2 and 4 were not statistically different (55.56 RVU and 58.22 RVU, respectively). Peak time, the time taken to reach peak viscosity, was longest at Site 1 (6.10 minutes) and shortest at Sites 2 and 3 (5.93 and 5.90 minutes, respectively), with Site 4 recording a peak time of 6.05 minutes. Pasting temperature, the temperature at which starch granules begin to swell and gelatinize, did not show significant differences among the sites, ranging from 78.40 °C to 79.48 °C.

Thermal properties

The thermal properties of Riceberry rice obtained from four distinct cultivation sites, as detailed in Table 5, include measurements of onset temperature (To), peak temperature (Tp), conclusion temperature (Tc), and the enthalpy of gelatinization (ΔH) for two specific peaks, determined by Differential Scanning Calorimetry (DSC). The result indicated the effect of the environmental factors on the gelatinization characteristics of Riceberry rice, and thus, changes in cooking characteristics and textural profile may be observed according to the geographical districts. The DSC curves presented in Figure 1 clearly explain the differences in gelatinization phenomena of rice starch obtained from each place.

Table 5. Thermal properties of organic Riceberry rice from four sites

Parameters	**Sample sites**			
	1	2	3	4
Peak 1				
To (ºC)	63.97 ± 0.56^{a}	62.57 ± 0.34^{bc}	61.75 ± 0.38^{c}	63.58 ± 0.47^{ab}
Tp (ºC)	71.65 ± 0.08^{a}	70.43 ± 0.19^{c}	69.16 ± 0.20^{d}	70.73 ± 0.11^{b}
Tc (ºC)	86.11 ± 0.27^{b}	84.77 ± 0.39^{c}	81.59 ± 0.43^{d}	87.64 ± 0.37^{a}
ΔH (J/g)	2.12 ± 0.09^{a}	2.12 ± 0.08^{a}	1.87 ± 0.07^{b}	1.68 ± 0.06^{c}
Peak 2				
To (ºC)	131.31 ± 0.24^{a}	30.47 ± 0.19^{bc}	130.83 ± 0.10^{ab}	130.33 ± 0.10^{c}
Tp (ºC)	131.50 ± 0.21^{ab}	131.69 ± 0.29^{a}	131.05 ± 0.13^{b}	130.49 ± 0.08^{c}
Tc (ºC)	139.65 ± 0.44^{a}	136.13 ± 0.43^{b}	136.54 ± 0.82^{b}	138.57 ± 0.40^{a}
ΔH (J/g) ^{NS}	1.56±011	1.42±0.21	1.48±0.07	1.61±0.29

Values are expressed as mean ± standard deviation (n = 3). Means with different letters in the same row were significantly different at the level $p < 0.05$ and NS = not significant. To: (onset of gelatinization temperature), Tp (peak of gelatinization temperature), Tc: (conclusion of gelatinization temperature), and ΔH (enthalpy of gelatinization).

Concerning the first peak related to the gelatinization process, the onset temperature (To) value, that is the temperature at which swelling of the starch granules and water uptake commences, ranged between 61.75 °C (Site 3) to 63.97 °C (Site 1). Site 1 showed the highest To value, while Site 3 had the lowest. Sites 2 and 4 exhibited intermediate values of 62.57 °C and 63.58 °C, respectively, with significant differences among the sites ($p < 0.05$). At site 3, the peak temperature (Tp) had the lowest value at 69.16 °C, while the highest value at site 1 was 71.65 °C. Here, we noted that Site 1 had the overall highest Tp value, while Site 3 had the lowest value of Tp. Sites 2 and 4 had Tp values of 70.43 °C and 70.73 °C, respectively, with significant differences among all sites ($p < 0.05$). The highest value of conclusion temperature (Tc), carrying the highest degree of gelatinization, was highest at Site 4 (87.64 °C), and lowest at Site 3 (81.59 °C). Sites 1 and 2 showed the Tc values of 86.11 °C and 84.77 °C, respectively, with significant differences observed among all sites ($p < 0.05$). The enthalpy change (ΔH), which depicts the energy for the gelatinization process, reached its maximum at Sites 1 and 2 with 2.12 J/g, while the minimum value of 1.68 J/g was obtained at Site 4. For Site 3, its value was intermediate, equal to 1.87 J/g the values vary significantly between the sites ($p < 0.05$).

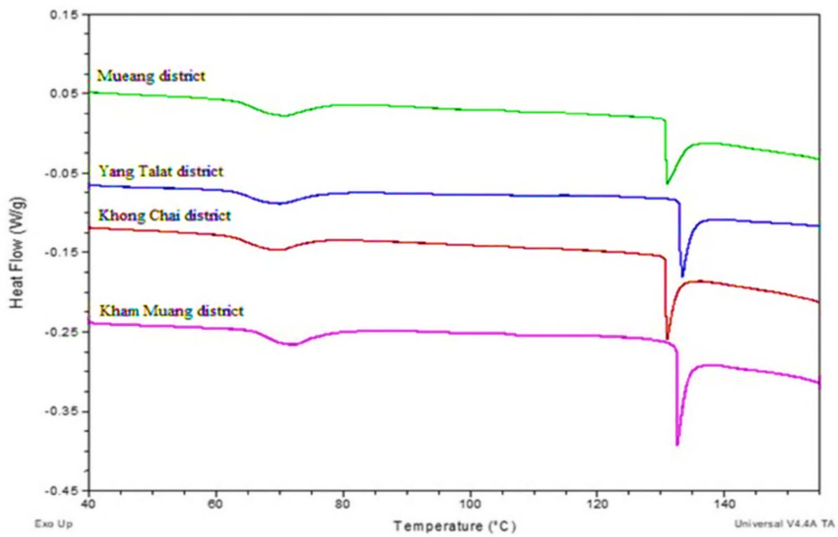

Figure 1. Differential scanning calorimetry curves of organic Riceberry rice from four sites

For the second peak of the thermal profile, corresponding to starch retrogradation, the To values were reported to vary between 130.33 °C (Site 4) to 131.31 °C (Site 1). Site 1 had the highest To, followed by Sites 3 (130.83 °C) and 2 (130.47 °C), with significant differences among the sites ($p < 0.05$). The Tp values for the second peak, indicating the maximum retrogradation temperature, ranged from 130.49 °C (Site 4) to 131.69 °C (Site 2), with significant differences among the sites ($p < 0.05$).

Discussion

The physical and chemical characteristics of rice plants are influenced by the drainage and texture of the soil, which has a major impact on water availability and nutrient uptake. For instance, Site 1's coarse-textured, poorly drained soils probably restrict the absorption of water and nutrients, leading to a lower lipid and protein content than other locations. On the other hand, the moderate amylose content (13.23%) and greater antioxidant properties of Site 2's better-drained soils indicate that, even with their significant acidity, they may promote more effective nutrient absorption (Xu *et al.,* 2015).

Studies have indicated that the physicochemical characteristics of rice are directly influenced by soil factors, including drainage and texture. Due to restricted oxygen and nutrient availability, poor drainage can reduce lipid content and synthesis of starch (Sharma *et al*., 2014). On the other hand, as Sites 2 and 4 showed higher amounts of protein and crude fiber, well-drained soils improve nutrient transmission and may therefore increase lipid and protein content.

In comparison to the other sites, the acidic soils at Site 2 exhibit noticeably greater levels of antioxidant activities, such as DPPH, FRAP, and TPC. Minerals and elements that act as a base for antioxidants such as phenolics and flavonoids can be more available in acidic soil environments (Valencia Pérez *et al*., 2023). This results in increased phenolic content and enhanced antioxidant ability.

Chemical compositions, the moisture content was not statistically different, indicating that environmental conditions or post-harvest handling practices were similar for all sites. The variation of lipid content may be caused by the differences in some soil characteristic that affects lipid synthesis by the rice grains including the organic matter content and fertility levels of the soils. Literature review indicates that rice with higher organic matter in its source of production contains higher lipid content due to improved nutrient availability as reported by Arslan *et al*. (2016). For the protein content, the difference could be attributed to one of the most important factors that influence protein synthesis that is the nitrogen supply in the soil. Rice grains, which are grown in soils with the best pH as well as suitable levels of nitrogen, tend to contain higher proportions of protein (Moongngarm and Saetung, 2010). Differences in crude fiber content might be due to physical and chemical attributes of the soil incorporating texture and organic matter influencing the growth and development of the rice plant which include of husk as well as bran (Butsat and Siriamornpun, 2010). The variation of the total mineral content or ash content may be as a result of variation in the concentration of the soil minerals or the efficiency of the rice plants in the process of mineral uptake. Higher ash content at Site 4 reveals richer soil mineral composition or better conditions for mineral absorption (Heinemann *et al*., 2005). No significant differences of the carbohydrate content indicate that carbohydrate accumulation in Riceberry rice is likely not strongly affected by the environmental or soil conditions in these regions. Differences in the amylose content are among the factors that possibly cause changes in the texture of the rice when cooked. The proportion of amylose in the rice indicated that Site 3 samples had higher amylose content, which tends to give hard and non-sticky rice while low amylose gives a softer and sticky rice. Environmental factors such as temperature during grain filling can significantly influence amylose synthesis (Yu *et al*., 2012).

Antioxidant properties, the contrasts between the four sites imply that the rice's antioxidant substances are impacted by the local environment, including the soil type, temperature, and sunlight. Higher antioxidant activities recorded at Sites 2, 3, and 4 can be explained in relation to enhanced environmental conditions suitable for the production of phenolic compounds and other bioactive compounds (Butsat and Siriamornpun, 2010). Research also indicates that environmental factors such as the intensity of solar radiation and soil type might affect phenolic acid, flavonoid, and anthocyanin contents of rice, which are responsible for antioxidant activity (Goufo and Trindade, 2017). Significantly higher TAC value in Site 4 than in Site 1 shows that environmental conditions of Site 4 were more favorable for anthocyanin synthesis, for example, lower temperature during the grain maturation or higher UV radiation that is known to act as strong inducer of anthocyanin biosynthesis (Jumrus *et al*., 2022). These findings highlight that environmental factors play a crucial role in determining the antioxidant potential of Riceberry rice. Moreover, recent advances demonstrate that ultrasonic-assisted enzymatic pretreatment can effectively disrupt bran cell walls, thereby enhancing the release of bound phenolic compounds and antioxidants, which improves bioaccessibility and elevates the nutritional quality of rice-based functional foods (Chamsai and Wanyo, 2025).

The variations in pasting properties of the four sites first provide evidence that starch properties of Riceberry rice are affected by the environment, mainly soil and climate factors. For instance, Site 4, which depicted the highest peak viscosity, the lowest trough, and the final viscosity may contain conditions favorable to the production of rice with a higher starch content or with more intact granules that swell during heat treatment. Such characteristics are often synchronized with improved water absorption and increased viscosity at the time of cooking depending on the soil moisture and nutrient availability (Juliano and Tuaño, 2019). This means that variations in peak viscosity imply differences in the amylose-to-amylopectin ratio of the various rice samples. It is as well seen that higher amylopectin content that causes higher swelling of starch granules leads to a higher peak viscosity, while higher amylose content, although causing lower peak viscosity, leads to higher setback viscosity as observed at Site 3. This may mean that there is a higher possibility for retrogradation, which may have consequences on the texture and firmness of cooked rice (Tan *et al*., 2021).

The differences observed for thermal properties indicate that other factors, such as soil type, temperature, and moisture content, impact the structure of starch in Riceberry rice. The starches in rice from Site 1 showed higher onset peak, and conclusion temperatures, which means that more energy is required to gelatinize the starches present in the samples, perhaps due to a higher proportion of crystalline structures or more compact granules. This could be due to soil properties that facilitate compact folding of the starch granules, making them shrink and become resistant to swelling and gelatinization (Wang *et al*., 2022). The enthalpy of gelatinization (ΔH) values explains the stability of the granules and structure of starch. Higher ΔH at sites 1 and 2 means that more energy is needed to gelatinize the starch granules, meaning that the granules are more stable, and could be due to high amylase contents or stronger forces within the granules. On the other hand, Site 4 has a lower ΔH, which suggests that the starch granules are less stable and gelatinize more easily; this could be because the grains contain high amounts of amylopectin or have weaker structural integrity (Zhu, 2018). Furthermore, the retrogradation properties, which are represented by the second peak temperatures and ΔH, are the results of restacking and reorganization of starch molecules, particularly amylose molecules. The higher retrogradation temperature observed at Site 1 may be as a result of higher amylose content, which is believed to cause a higher degree of retrogradation leading to the formation of a firmer texture in cooled rice products, as observed by Chung *et al*. (2009).

This study investigated the physicochemical properties, antioxidant activities, pasting characteristics, and thermal properties of Riceberry rice from four different sites in Thailand (Mueang, Yang Talat, Khong Chai, and Kham Muang districts). Differences in lipid, protein, fiber, ash, and amylose are explained by the ability of rice plants to adapt to local soil and environmental conditions to absorb and incorporate nutrients. For antioxidant activities expressed as DPPH, FRAP, TPC, TFC, and TAC assays, the values obtained were higher for the sites with an environment that favors the biosynthesis of phenolic compounds and flavonoids, namely an acidic pH of the soil and moderate availability of water. This means the pasting properties such as peak viscosity, trough viscosity, breakdown, and final viscosity, as well as setback values, presented different trends in the four sites, potentially due to environmental factors affecting the amylose/amylopectin ratio such as fertility and soil moisture.

Thus, the case and findings of the study indicated that improvement of the environmental factors, including the soil and irrigation, can improve the nutritional and functional quality of the organic Riceberry rice. For instance, rice produced from sites that contain higher antioxidant activities and better pasting and thermal stability could be branded as health food with improved cooking qualities. Further studies should focus on identifying the pathways of bioactive compounds biosynthesis in Riceberry rice under different environmental conditions and studying genetic factors that resulted in differences in rice properties at the different sites.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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