Starch Citrate Synthesis from Diverse Thai Rice Cultivars: A Study on Resistant Starch Enhancement and Physicochemical Changes.

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Abstract. This study investigates the synthesis and characterization of starch citrate derived from five Thai rice cultivars: Rice, Riceberry, Homnil, Red Jasmine, and Thubtim Chumphae using citric acid esterification. The objective was to enhance resistant starch (RS) content and assess changes in structural and functional properties. Native rice starches were chemically modified and evaluated through resistant starch assays, Fourier-transform infrared spectroscopy (FTIR), Rapid Visco Analyzer (RVA), scanning electron microscopy (SEM) and water absorption index. Results showed a significant increase in RS content in citrate-modified flours, with white rice exhibiting the highest RS value (55.04%). FTIR confirmed ester bond formation through the appearance of C=O stretching bands at 1734–1740 cm^{-1}, SEM images revealed pronounced granule surface disruption post-modification. The physicochemical changes confirm successful citric acid crosslinking, which improves starch resistance to enzymatic digestion. These findings highlight the potential of citric acid esterification in developing functional starches with health-promoting properties and enhanced thermal and morphological characteristics.

1 Introduction

Rice (*Oryza sativa L.*) is a vital staple crop worldwide and a major source of starch used extensively across food, pharmaceutical, and industrial sectors. Thailand is home to a wide array of rice cultivars, each exhibiting distinct physicochemical properties that influence their functional applications.

A key focus of this research is resistant starch (RS), a form of starch that escapes digestion in the small intestine and undergoes fermentation in the large intestine, where it contributes to gut health, glycemic regulation, and the production of beneficial short-chain fatty acids. RS is classified into five types based on its origin and structural resistance to enzymatic breakdown: RS1, physically protected starch found in whole grains and seeds; RS2, native granular starch with crystalline structure such as in raw potatoes and unripe bananas; RS3, retrograded starch formed upon cooling of gelatinized starch; RS4, chemically modified starch produced through reactions like esterification or etherification; and RS5, complexes of amylose and lipids that resist digestion. Among these, RS4, particularly through citric acid modification, offers promising potential for developing functional starches with enhanced health benefits. Various processes exist to produce RS; each suited to different applications. Conventional hot air oven treatment (120–160°C) induces starch reorganization to form RS [1] Autoclaving uses high temperature and pressure to promote retrogradation, enhancing resistance [2]. Stirred reactors allow controlled modification of starch digestibility through precise regulation of temperature and pressure. Extrusion is widely employed in large-scale RS production due to its efficiency and precise process control [4, 5]. Compared to methods such as autoclaving, extrusion, stirred-tank bioreactors, or microwave treatment, the conventional hot air oven method offers a convenient, easy, and promising approach for producing starch citrate.

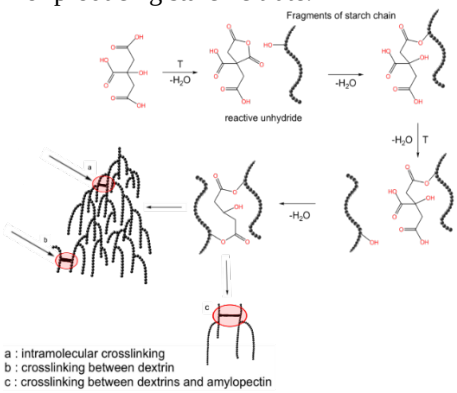

Fig. 1. Mechanism and molecular structure of starch esterification by citric acid

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Native starch granules consist of crystalline and amorphous regions: crystalline areas mainly formed by amylopectin double helices, while amylose and amylopectin intertwine in amorphous zones. Differences in crystalline structure, polymerization degree, and amylose-to-amylopectin ratio among starch sources affect their physicochemical properties. During esterification, citric acid crosslinks starch molecules by forming anhydride bonds upon heating, which react with hydroxyl groups to produce mono- and diesters. This crosslinking creates intramolecular (Type A) and intermolecular (Types B and C) linkages, increasing structural rigidity and reducing solubility in starch citrate (Figure 1). Among chemical modifications, esterification of native starch with citric acid is well-documented [6-9]. Industrially, citrate is primarily produced by fermentation and utilized as a food additive (E330) for its acidity-modifying and antioxidant properties. Citrate-modified starches exhibit enhanced resistant starch content and potential functional benefits such as hypoglycaemic effects, making them attractive ingredients in functional foods, as well as in non-food applications like textile dressings and drilling fluids [6].

Given the rising interest in RS as a functional ingredient for health-promoting foods, this study evaluates the modification of starch from selected Thai rice cultivars via citric acid esterification and investigates the resultant structural and functional properties, with an emphasis on resistant starch content and gelatinization behaviour. These cultivars were modified by esterification with citric acid to produce starch citrate, followed by characterization of resistant starch content and structural properties via FTIR, RVA, water absorption index and SEM.

2 Materials and methods

2.1 Chemicals

Five Thai rice cultivars were used to study the gelatinization. Rice flour (*Oryza sativa L.*), Riceberry rice (*Oryza sativa L.*), Homnil rice (*Oryza sativa var. indica*), Red jasmine rice (*Oryza sativa var. indica*), and Thapthim Chumphae rice (*Oryza sativa var. indica*) were obtained from Nana Rice, (Bangkok, Thailand). Citric acid and sodium bicarbonate were purchased from the Chemipan Cooperation Co., Ltd (Bangkok, Thailand). Resistant starch assay kit was brought from Megazyme International Ireland Ltd., (Wicklow, Ireland). All other chemicals and solvents were of analytical grade.

2.2 Starch preparation

A starch was prepared by milling five Thai rice varieties using a SUS304 grinder (Vientian Tool, China). The resulting rice flour was then subjected to particle size separation by sieving, using a 125-mesh sieve, to obtain flour with uniform particle size suitable for further processing.

2.3 Characterization of starch: pasting viscosity

The pasting properties of five Thai rice starch varieties: Rice flour, Riceberry, Homnil rice, Red Jasmine rice and Thapthim Chumphae rice were determined using a Rapid Visco Analyser (RVA-3D; Newport Scientific, NSW, Australia). Starch samples (2.24 g, dry basis) were weighed directly into RVA canisters, followed by the addition of distilled water to bring the total sample weight to 28 g. The starch suspensions were equilibrated at 50°C for 2 minutes, after which the temperature was increased to 95°C at a rate of 12°C/min and held at 95°C for 2 minutes. The temperature was then decreased back to 50°C at the same rate (12°C/min) and held for an additional 2 minutes. Throughout the analysis, the paddle rotated at a constant speed of 160 rpm. Pasting parameters such as peak viscosity, breakdown, setback, and final viscosity were recorded and used to characterize the starch pasting behaviour.

2.4 Preparation of starch citrate using a convection drying

Citrate starch was synthesized by esterification with citric acid. A 20% (w/v) citric acid solution was prepared by dissolving citric acid in distilled water, adjusting the pH to 3.5 using 10 M NaOH, and diluting to a final volume of 1000 mL. Then, 1000 mL of this solution was thoroughly mixed with 1000 g of native rice starch. The mixture was incubated in a hot-air oven at 50°C for 16 hours to allow for initial absorption and equilibration. After incubation, the sample was finely ground, and its moisture content was adjusted to approximately 10% (w/w). The starch was then subjected to a thermal reaction by heating at 160°C for 5 hours to promote ester bond formation. Following thermal treatment, the product was ground again and passed through a 125-mesh sieve. To remove unreacted citric acid, the modified starch was washed four times with absolute ethanol, followed by a final wash with 1 M sodium bicarbonate until the pH of the wash solution reached approximately 7. The resulting starch was then air-dried at 45°C and passed through a 125-mesh sieve to obtain citrate-modified starch in powder form.

2.5 Characterization of starch citrate

2.5.1 Resistant starch contents

The resistant starch (RS) content of starch citrate was determined using a commercial Resistant Starch Assay Kit (K-RSTAR; Megazyme Ltd., Australia), following the manufacturer's protocol. Finely ground starch citrate samples (100 ± 5 mg) were weighed into capped test tubes. To each tube, 4.0 mL of a freshly prepared enzyme mixture containing pancreatic α-amylase and amyloglucosidase (AMG) was added. The mixture was vortexed and incubated at 37°C in a shaking water bath at 200 rpm for 16 hours to allow enzymatic hydrolysis of digestible starch. Following incubation, 4.0 mL of 99% ethanol was added to each tube to precipitate the resistant starch, and samples were centrifuged at 1,500× g for 10

minutes. The resulting pellet was washed twice with 50% (v/v) ethanol, followed by centrifugation under the same conditions. The pellet was then resuspended in 2.0 mL of 2 M KOH and stirred in an ice bath for 20 minutes to solubilize the resistant starch. Afterward, 8.0 mL of 1.2 M sodium acetate buffer (pH 3.8) and 0.1 mL of AMG were added, and the mixture was incubated at 50°C for 30 minutes to hydrolyze the solubilized starch into glucose. The sample was centrifuged again at 1,500 × g for 10 minutes, and the supernatant was collected for glucose determination. Glucose content was quantified calorimetrically using the glucose oxidase-peroxidase (GOPOD) reagent, with absorbance measured at 510 nm using a spectrophotometer (Molecular Devices, USA). Resistant starch content was calculated based on the amount of glucose released.

2.5.2 Fourier transform infrared spectroscopy (FTIR)

Pulverized and dried samples (10 mg) were analyzed using Fourier Transform Infrared (FTIR) spectroscopy in attenuated total reflectance (ATR) mode with an INVENIO S spectrometer (BRUKER, Germany). Spectra were recorded in the range of 4000–400 cm^{-1} with a spectral resolution of 2 cm^{-1}, averaging 25 scans per sample to enhance signal quality. The degree of crystallinity was assessed by calculating the crystallinity index using Equation (1), based on the absorbance ratio of characteristic peaks at 1047 cm^{-1} (crystalline region) and 1022 cm^{-1} (amorphous region):
Crystallinity of peaks was calculated using Eq. (1)

$$\text{Crystallinity} = \frac{A_{1047}}{A_{1022}} \qquad (1)$$

This method of crystallinity estimation is based on the approach described by Liu, Mingyue, et al. [10]

2.5.3 Scanning electron microscopy (SEM)

The surface morphology of starch citrate was examined using a scanning electron microscope (JEOL JSM-IT500HR, Japan). Samples were mounted onto aluminum stubs using double-sided conductive carbon tape. Imaging was conducted at an accelerating voltage ranging from 3.0 to 5.0 kV. SEM micrographs were captured at a magnification of 5,000× to observe surface features and particle morphology.

2.5.4 Determination of Water Absorption Index (WAI)

The WAI were determined based on the method described by [11]. 0.1 g of rice flour was mixed with 10 mL of distilled water and vortexed for 1 minute. The mixture was then gently stirred and incubated in a water bath at 30°C for 30 minutes. After incubation, the samples were centrifuged at 3000 rpm for 10 minutes. The WAI were calculated using the following equations:

$$\text{WAI (g/g)} = \frac{\text{Weight of wet sediment (g)}}{\text{Dry weight of flour (g)}} \qquad (2)$$

Each analysis was performed in triplicate, and the results are expressed as mean values with standard deviations.

2.5.5 Determination of Moisture Content

The moisture content of flour was determined using a moisture analyzer (Shimadzu MOC-63u, Japan) based on the thermogravimetric method. Approximately 2 g of sample was evenly spread on the aluminum sample pan and analyzed at 105°C using automatic mode. The result was recorded as percentage moisture content (%MC) when the weight loss stabilized. Each sample was measured in triplicate.

2.5.6 Determination of Moisture Content

The total phenolic content was determined using a modified Folin–Ciocalteu method [12]. Briefly, 200 µL of rice extract was mixed with 1.0 mL of freshly prepared and diluted (1 : 10, v/v) Folin–Ciocalteu reagent. The mixture was vortexed for 1 minute, followed by the addition of 800 µL of 10% (w/v) sodium carbonate (Na_2CO_3) solution. After mixing thoroughly, the reaction mixture was incubated at room temperature for 2 hours. The volume was adjusted to 5.0 mL with distilled water. Absorbance was measured at 760 nm using a UV–Visible spectrophotometer. The total phenolic content was calculated from a gallic acid standard curve and expressed as milligrams of gallic acid equivalents per 100 g of dry weight (mg GAE/100 g DW).

2.6 Statistical analysis

The analytical experiments were conducted in triplicate. The SPSS 22.0 software (Chicago, IL, USA). was employed to conduct an ANOVA analysis of the results. The Tukey's test was adopted to conduct multiple comparisons of the means at a significance level of 0.05.

3 Results and discussion

3.1 Pasting viscosity

In this study, a distinct difference in color was observed between native rice flour and citrate-modified rice flour, as shown in Figure 2. The native flours, derived from five Thai rice cultivars—Riceberry, Homnil, Red Jasmine, Thapthim Chumphae, and standard white rice—exhibited a range of natural hues that closely resembled the pigmentation of their corresponding rice grains. However, after modification with citric acid under thermal treatment, all samples showed significantly darker coloration. This was particularly evident in flours from pigmented rice varieties such as Riceberry and Red Jasmine, which changed from light greyish or purplish tones to deep brown or golden shades. The notable color transformation is primarily attributed to the Maillard reaction, a non-enzymatic browning process that occurs

when reducing sugars react with free amino acids or proteins under high-temperature conditions. This reaction results in the formation of melanoidins, which are irreversible brown polymers responsible for the dark coloration [13, 14]. In this case, the citric acid modification was carried out at 160°C, a temperature conducive to efficient Maillard reaction activity [15]. Pigmented rice varieties, particularly those rich in phenolic compounds such as anthocyanins, flavonoids, and tannins—as well as higher levels of proteins and free amino acids compared to white rice—serve as ideal precursors for accelerating browning reactions [16, 17].

The pasting properties of starches derived from five Thai rice cultivars: Rice flour, Riceberry, Homnil, Red Jasmine, and Thapthim Chumphae were evaluated using a rapid visco analyzer to understand their gelatinization behavior and functional characteristics. Among the samples as shown in Figure1 and results in Table 1, native rice starch exhibited the highest peak viscosity (189.17 cP), indicating strong swelling and water absorption capacity during heating. Red Jasmine rice followed with a peak viscosity of 160.58 cP, while Homnil (94.83 cP), Thapthim Chumphae (88.92 cP), and Riceberry (50.00 cP) showed progressively lower values. The breakdown viscosity, which reflects the stability of the starch granules under heat and shear, was greatest in Red Jasmine rice (43.00 cP), suggesting moderate structural integrity, whereas Thapthim Chumphae displayed the lowest breakdown (5.58 cP), indicating higher stability. Final viscosity, representing the viscosity after cooling, was highest in Rice flour (325.17 cP), indicating significant retrogradation tendency, while Riceberry had the lowest (38.58 cP), reflecting limited ability to reassociate. Setback viscosity, the difference between final and trough viscosity, was also highest in Rice (169.50 cP), signifying greater retrogradation, and lowest in Riceberry (11.42 cP). Peak times ranged from 5.87 to 6.47 minutes across all samples, with Thapthim Chumphae starch requiring the longest time to reach peak viscosity. The pasting temperature varied between cultivars, from 73.5°C in Riceberry to 83.0 °C in Thapthim Chumphae, reflecting differences in granular resistance to swelling and gelatinization. The peak time and pasting temperature parameters reflect the kinetic and thermal properties of starch gelatinization. Thubtim Chumphae rice required the longest time to reach peak viscosity (6.47 min) and had the highest pasting temperature (83°C), indicating slower gelatinization kinetics and higher thermal stability, possibly due to variations in starch granule size or protein matrix effects [18]. These findings reveal distinct pasting profiles among the rice starches, emphasizing the influence of cultivar-specific properties on starch functionality and modification potential. Collectively, these RVA profiles highlight significant varietal differences in starch pasting behaviour, with implications for processing and product development in the rice industry. The understanding of these physicochemical properties is crucial for tailoring rice-based products with desired textural and functional attributes [19].

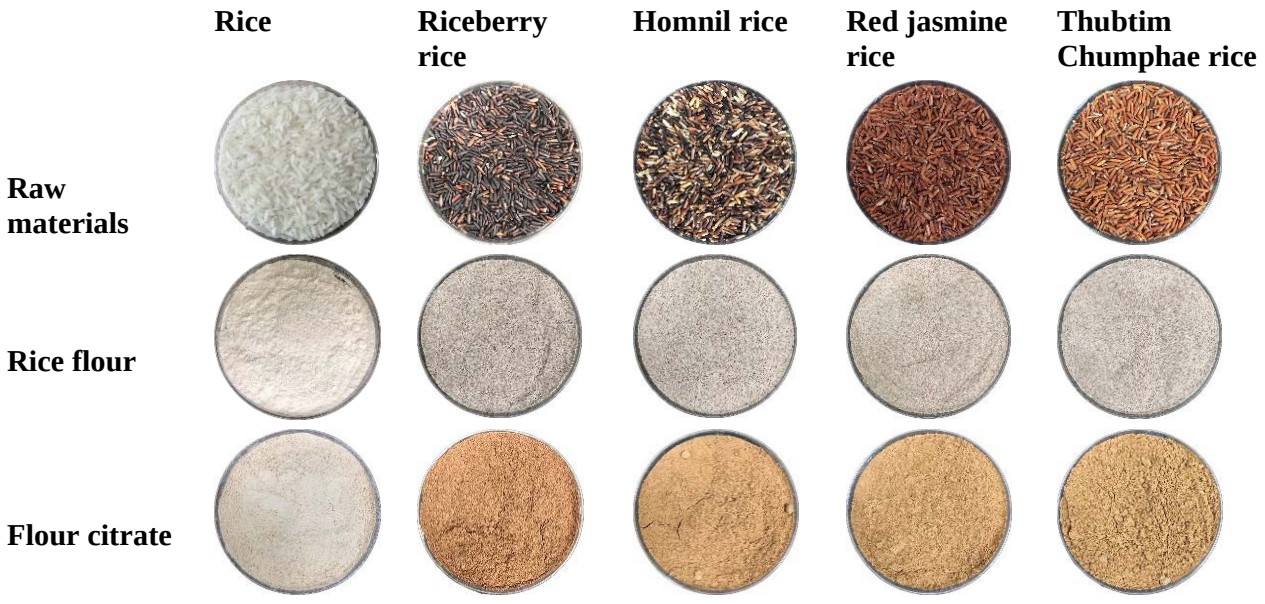


Fig. 2. Comparison five Thai rice of native and flour citrate.

Table 1. Pasting viscosity parameters of Thai rice.

Sample	Pasting viscosity parameters						
	Peak viscosity (cP)	**Trough viscosity (cP)**	**Breakdown viscosity (cP)**	**Final viscosity (cP)**	**Setback viscosity (cP)**	**Peak time (min)**	**Pasting temperature (° C)**
Rice flour	189.17	155.67	33.5	325.17	169.5	6.33	79.1
Riceberry Rice	50	27.17	22.83	38.58	11.42	3.73	73.5
Homnil rice	94.83	80.08	14.75	158.92	78.83	6	76.7
Red jasmine rice	160.58	117.58	43	260.17	142.58	5.87	82.3
Thubtim Chumphae rice	88.92	83.33	5.58	151.42	68.08	6.47	83

Different letters indicate statistically significant differences ($p < 0.05$)

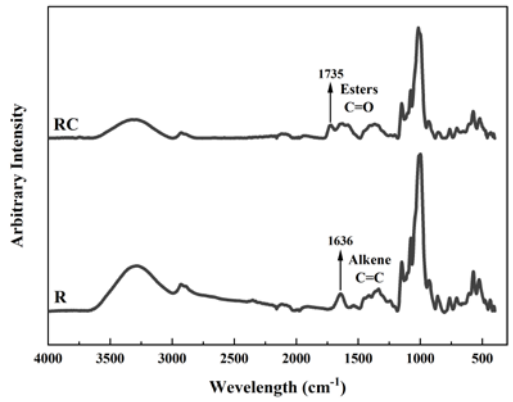
3.2 Resistant starch contents

The resistant starch content of modified starch citrate derived from five Thai rice cultivars varied significantly, reflecting the impact of botanical source and modification efficiency. As shown in Table 2, rice starch citrate showed the highest resistant starch (RS) content at 55.04%**,** followed by Thapthim Chumphae **(**46.49%**),** Riceberry **(**46.34%**),** Red Jasmine **(**45.82%**),** and Homnil rice (45.47%). These values were markedly higher compared to the native starch control, which exhibited only 9.22%, confirming the effectiveness of citric acid esterification in enhancing starch resistance to enzymatic digestion The resistant starch (RS) content of native flours derived from different rice varieties exhibited significant variation, with white rice (*Oryza sativa*) demonstrating the highest RS content at 55.04%. This finding aligns with previous studies reporting elevated RS levels in native starches extracted from specific rice cultivars due to their unique amylose to amylopectin ratios and granular structures [20, 21]. In contrast, the RS content in pigmented rice varieties such as Riceberry, Homnil, Red Jasmine, and Thubtim Chumphae ranged from 45.47% to 46.49%, consistent with findings by [22], who noted lower RS in pigmented rice attributed to differences in starch granule morphology and the presence of phenolic compounds.

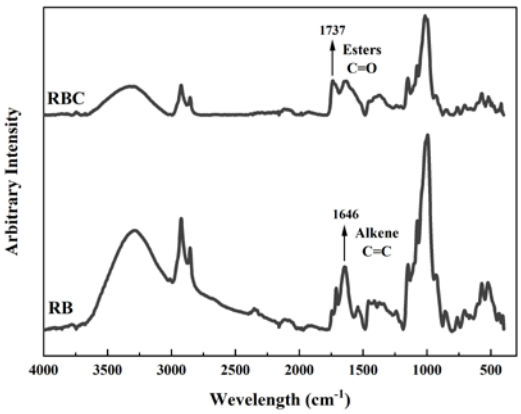
Table 2. Resistant starch content (%) of native and starch citrate.

Sample	Native		modified	
	Resistance starch content (%)	**Moisture content (%)**	**Resistance starch content (%)**	**Moisture content (%)**
Rice flour	1.11±0.19[a]	12.47±0.35[a]	55.04 ± 0.61[b]	12.50 ± 1.04[a]
Riceberry rice	0.96±0.11[a]	1.54±0.71[a]	46.34 ± 0.35[b]	1.88 ± 0.69[a]
Homnil rice	0.27±0.03[a]	1.59±1.37[a]	45.47 ± 0.35[b]	2.15 ± 0.34[b]
Red jasmine rice	0.13±0.01[a]	2.28±0.27[a]	45.82 ± 0.67[b]	2.35 ± 0.31[a]
Thubtim Chumphae rice	0.14±0.10[a]	4.78±1.07[a]	46.49 ± 0.37[b]	4.78 ± 0.27[a]

3.3 FTIR spectra of native and flour citrate



(a)



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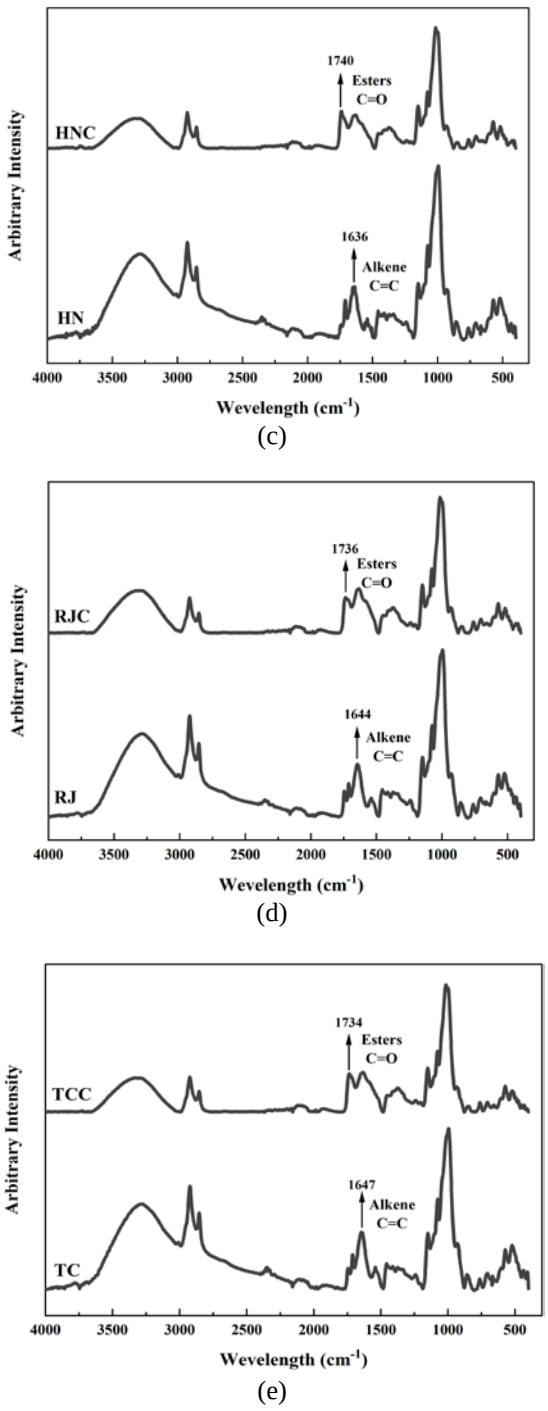


Fig. 3. FTIR spectra of native and flour citrate: (a) Native flour Rice (R) and Rice citrate (RC), (b) Riceberry rice (RB) and Riceberry rice citrate (RBC), (c) Homnil rice (HN) and Homnil rice citrate (HNC), (d) Red jasmine rice (RJ) and Red jasmine rice citrate (RJC), and (e)Thubtim Chumphae rice (TB) and Thubtim Chumphae rice citrate (TBC)

Table 3. Crystallinity Index from fourier transform infrared spectroscopy (FTIR)

Sample	Crystallinity Index	
	Native	Modified
Rice flour	0.62	0.58
Riceberry rice	0.74	0.65
Homnil rice	0.72	0.62
Red jasmine rice	0.71	0.62
Thubtim Chumphae rice	0.73	0.64

Fourier Transform Infrared (FTIR) analysis of native and citric acid-modified rice flours revealed significant changes in the chemical composition and structure of the starch molecules after modification. All modified rice flours exhibited an absorption band at 1734ó1740 cm^{-1}, which was absent from native flours (with some exceptions). This band corresponds to the carbonyl bond (C=O) vibration of the ester group, suggesting the formation of an ester bond between the hydroxyl group of starch and the carboxyl group of citric acid. This confirms the success of the chemical modification via esterification [1 ,5 ,6 ,27] , which affects functional properties of the starch, such as its resistance to digestion (resistant starch type 4 : RS4).

Furthermore, the absorption band at 1636-1647cm^{-1}, associated with the C=C bond vibration or water bending (H–O–H bending), was reduced in the modified starches compared to the native flours. This reflects a change in molecular structure or a decrease in unsaturated substances in the starch that may decompose under acidic conditions [17, 18].

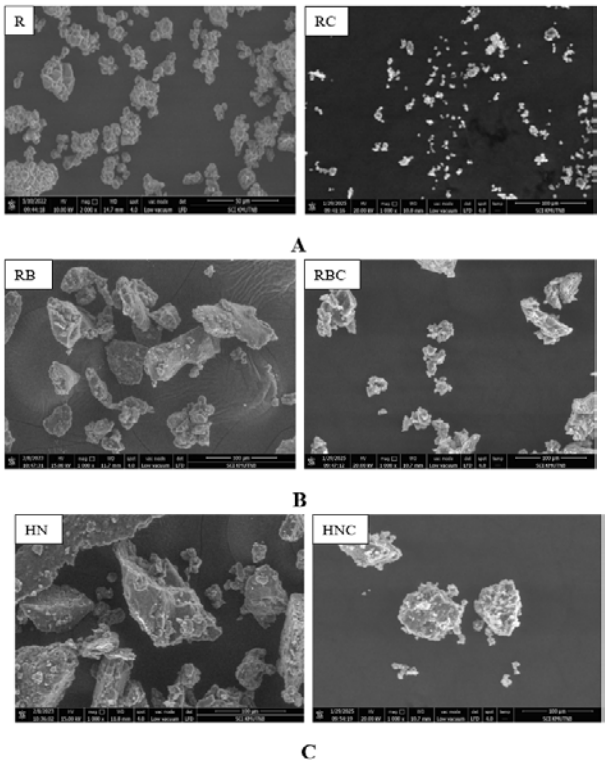
Meanwhile, the Crystallinity Index (CI) of all rice flours decreased significantly after modification, indicating a partial loss of crystal structure and an increase in amorphous regions, which may promote starch swelling, water solubility, and resistance to digestion. In particular, Hom Nil rice flour exhibited the highest CI reduction (0.10) , followed by Riceberry, Hom Mali Daeng, and Tubtim Chumphae rice (0.09 reduction). White rice flour exhibited only a 0.04 reduction [1, 3, 6] . Also, a weak absorption band was observed at approximately 1735 cm^{-1} in the flours of native rice, Riceberry, Hom Mali Daeng, and Tubtim Chumphae, even without modification. This is different from white rice flour, which lacks this signal at all. This is due to the presence of fatty or phenolic compounds with natural ester groups in unmilled rice varieties, which can provide a signal in this range. As white rice undergoes intensive milling, these compounds are removed [16, 17].

Furthermore, the O–H stretching bands in the 3100-3600 cm^{-1} region, associated with hydroxyl groups, decreased in intensity in the modified starch, reflecting the partial replacement of hydroxyl groups with ester groups by chemical reactions. Meanwhile, the fingerprint region (900ó1200 cm^{-1}), particularly at 1040 and 1020 cm^{-1}, exhibited changes in the intensity of the absorption bands, indicating changes in the structure of glycosidic bonds and the polysaccharide backbone, which are associated with intramolecular crosslinking of the starch molecules [6, 21, 27].

Overall, the FTIR analysis results indicated significant structural changes at the chemical bond level due to the citric acid modification process.

3.4 Scanning electron microscopy

The morphological characteristics of native and citrate-modified rice flours were examined using scanning electron microscopy (SEM), as shown in Figure 4. Native flours from all rice varieties: Rice (R), Riceberry (RB), Homnil (HN), Red Jasmine (RJ), and Thubtim Chumphae (TC) exhibited irregular, angular granules with well-defined edges and compact, smooth surfaces, indicative of intact starch granule structures. In contrast, their citrate-modified counterparts (RC, RBC, HNC, RJC, and TCC) showed significant structural disruption. The modified granules appeared more fragmented, with rougher surfaces, reduced size, and a more porous and eroded morphology. These changes suggest that citric acid treatment disrupted the semi-crystalline granular architecture through esterification and crosslinking, leading to granule disintegration and surface modification. The loss of structural integrity was especially evident in the modified samples, supporting the findings from FTIR that confirmed chemical modification and amorphization of the starch. [3, 25-27]. Overall, the SEM images illustrate that citric acid significantly alters starch granule morphology, which may impact the functional and physicochemical properties of the modified flours. Comparative analysis clearly indicates that citrate modification induces
extensive physical and structural changes to native starch granules, which can be correlated with altered physicochemical behaviors and potentially improved techno-functional attributes.



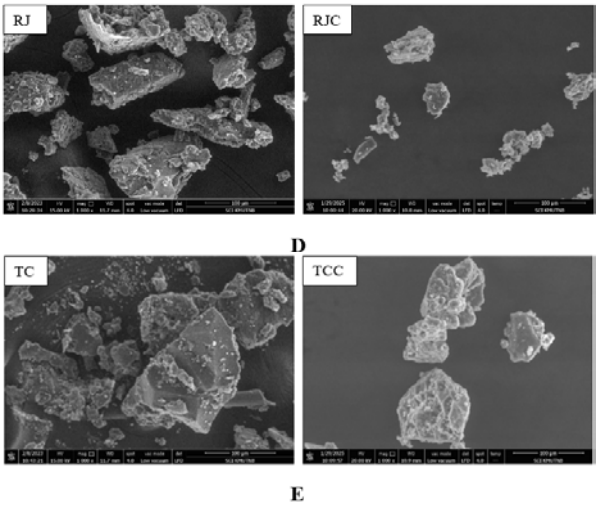


Fig. 4. SEM images of native and flour citrate: (a) Native flour Rice (R) and Rice citrate (RC), (b) Riceberry rice (RB) and Riceberry rice citrate (RBC), (c) Homnil rice (HN) and Homnil rice citrate (HNC), (d) Red jasmine rice (RJ) and Red jasmine rice citrate (RJC), and (e)Thubtim Chumphae rice (TB) and Thubtim Chumphae rice citrate (TBC)

3.5 Determination of Water Absorption Index (WAI)

In raw starch, the Water Absorption Index (WAI) tends to increase continuously with temperature due to the destruction of the starch crystal structure, allowing the starch to absorb more water [28, 30]. For example, red jasmine rice flour's WAI increased from 3.50 ± 0.10 at 30°C to 8.87 ± 0.16 at 70°C due to the breakdown of hydrogen bonds within the amylopectin molecule and partial dissolution of amylose, which increased the surface area for water absorption [28–30].

In contrast, when starch was crosslinked with citric acid, the WAI decreased significantly, especially at high temperatures. For example, modified riceberry flour's WAI decreased from 7.83 ± 0.06 to 3.27 ± 0.12 at 70°C [31–34]. This is due to citric acid's ability to form ester bonds with hydroxyl groups in the starch. This results in a stronger starch structure and limits swelling and water absorption [31, 32]. Especially at 70°C, the difference between raw and modified starches is clear. For example, raw red jasmine flour has a WAI of 8.87 ± 0.16 g/g, but after modification, it decreases to only 3.00 ± 0.19 g/g ($p<0.05$) [29, 32]. This is consistent with research indicating that crosslinked starches exhibit reduced heat resistance and water absorption due to their reinforced structure [29, 32–34].

These results indicate that at higher temperatures, native starch granules undergo extensive swelling due to the breakdown of crystalline structures and the disruption of hydrogen bonds, enabling greater water absorption. Meanwhile, citric acid-modified starches, which have undergone crosslinking, retain a more stable granular structure, thereby limiting swelling and water uptake even under heat. The chemical mechanism underlying this behavior is attributed to esterification or hydrogen bonding between the citric acid's carboxyl groups and the starch's hydroxyl groups. This results in a crosslinked network that reinforces the granule structure, enhancing thermal stability and reducing its ability to swell and

absorb water [32]. This finding aligns with the report by Singh et al. [29], which demonstrated that chemically crosslinked starches tend to exhibit lower WAI values due to their reinforced, compact structure that resists water penetration. In summary, citric acid modification effectively reduces excessive water absorption at elevated temperatures by enhancing the structural integrity of starch granules.

Table 4. Water Absorption Index (WAI) of flour and flour citrate

Sample	WAI (g/g) 30°C		WAI (g/g) 50°C		WAI (g/g) 70°C	
	Native	Modified	Native	Modified	Native	Modified
Rice flour	3.30±0.00^{a}	3.27±0.33^{a}	3.30±0.33^{a}	3.27±0.33^{a}	5.80±0.17^{b}	3.27±0.07^{a}
Riceberry rice	3.53±0.89^{a}	3.27±0.03^{a}	3.53±0.15^{a}	3.47±0.15^{a}	7.83±0.06^{b}	3.27±0.12^{a}
Homnil rice	3.53±0.09^{a}	3.47±0.15^{a}	3.87±0.33^{ab}	3.50±0.33^{a}	7.83±0.23^{c}	3.00±0.03^{a}
Red jasmine rice	3.50±0.10^{a}	3.47±0.15^{a}	4.1±0.20^{ab}	3.97±0.24^{ab}	8.87±0.16^{c}	3.00±0.19^{a}
Thubtim Chumphae rice	3.87±0.03^{a}	3.50±0.03^{a}	4.03±0.07^{a}	3.67±0.67^{a}	8.87±0.06^{b}	3.77±0.07^{a}

Different letters indicate statistically significant differences ($p < 0.05$)

3.6 Total Phenolic Content

Table 5. Total Phenolic Content of flour and flour citrate

Sample	Total phenol (mg/g)	
	Native	Modified
Rice flour	0.03±0.002^{a}	0.02±0.008^{a}
Riceberry rice	0.06±0.005^{a}	0.05±0.007^{a}
Homnil rice	0.05±0.001^{a}	0.04±0.001^{a}
Red jasmine rice	0.07±0.026^{a}	0.05±0.004^{a}
Thubtim Chumphae rice	0.06±0.011^{a}	0.05±0.005^{a}

Different letters indicate statistically significant differences ($p < 0.05$).

Total Phenolic Content (TPC) of native and citric acid-modified rice flours showed a decrease in phenolic content after modification, although the decrease was not statistically significant ($p > 0.05$) for all sample groups. For Riceberry, Hom Nil, Red Jasmine, and Tubtim Chumphae rice, the phenolic content of the modified flours decreased by approximately 0.01ó0.02 mg GAE/g compared to the native flours. White rice flour showed the lowest values for both the native ($0.03 \leq 0.002$ mg/g) and post-modification ($0.02 \leq 0.008$ mg/g) forms. The trend of phenolic content decrease may be due to the citric acid modification process, which requires high temperatures, resulting in the degradation or loss of some phenolic compounds. Furthermore, washing and drying processes may also leach some phenolic compounds from the flour. However, the measured TPC values still reflect the characteristics of each rice variety. In particular, colored rice such as Riceberry and Red Jasmine rice retained higher phenolic values compared to white rice even after modification, reflecting the potential of colored indigenous rice as a source of natural antioxidants.

4 Conclusion

This study demonstrates that citric acid modification significantly enhances the resistant starch (RS) content and alters the physicochemical properties of starches derived from various Thai rice cultivars. The increase in RS content is closely linked to the structural transformation of starch granules, particularly the interplay between amylose and amylopectin fractions. A higher amylose content typically promotes RS formation due to its linear structure that facilitates retrogradation and crystallization, while high amylopectin content favors a more branched and amorphous configuration. FTIR analysis confirmed the formation of ester bonds via C=O stretching bands and revealed a decrease in the crystallinity index across all modified starches. This suggests a disruption of the native crystalline regions—primarily formed by amylopectin double helices—and an increase in amorphous domains, which enhances the resistance of starch to enzymatic digestion. SEM images further supported this by showing granule surface erosion and increased porosity after modification, indicating granule disintegration and structural reorganization.

Additionally, the decreased water absorption capacity in citrate-modified starches highlights their reinforced structure due to crosslinking between citric acid and hydroxyl groups of starch. These structural changes result in improved mechanical integrity and thermal stability. In conclusion, the citric acid esterification process effectively modifies starch by modulating the amylose/amylopectin ratio's influence on crystalline versus amorphous regions, thereby enhancing RS content. This makes citrate-modified starches suitable for applications in functional foods, where resistance to digestion and controlled water interaction are desired.

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