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A comparative study of the modification of black glutinous rice starch using acid hydrolysis-autoclaving cooling and octenyl succinic anhydride

Akhmad Mustofa[1,2], Choiroel Anam[3], Danar Praseptiangga[3] and Sutarno[4*]

[1]Department of Doctoral Program of Science Agricultural, Faculty of Agriculture, Universitas Sebelas Maret, Surakarta 57126, Indonesia
[2]Department of Agricultural Technology, Faculty of Technology and Food Industry, Universitas Slamet Riyadi, Surakarta 57136, Indonesia
[3]Department of Food Science and Technology, Faculty of Agriculture, Universitas Sebelas Maret, Surakarta 57126, Indonesia
[4]Department of Biology, Faculty of Mathematics and Natural Science, Universitas Sebelas Maret, Surakarta 57126, Indonesia
*Corresponding author: nnsutarno@staff.uns.ac.id

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Abstract

Starch modification can alter its characteristics to align with industrial requirements. Modified starch can be developed as an alternative food product with the desired properties or characteristics. Modifying black glutinous rice starch using acid hydrolysis-autoclaving cooling (AH-AC) and esterification using octenyl succinic anhydride (OSA) represents a novel approach. This study examines the characteristics of black glutinous rice starch modified through the AH-AC method and esterification using OSA. In addition to amylose, amylopectin, dietary fibre, antioxidant activity and anthocyanin content, the modified starch was also examined for its swelling power and pasting properties, degree of substitution for OSA starch, and visual appearance. The findings indicate that starch modification altered the content of amylose, amylopectin and total-resistant starch. Additionally, the swelling power, peak viscosity (PV) and trough viscosity (TV) of OSA-modified starch were superior to those of the AH-AC version. The electron micrographs of the structure of the OSA and AH-AC-modified starches also differed. Scanning electron microscopy (SEM) of the modified starches revealed an uneven surface, wavy and nonpolygonal shape, and altered size.

Keywords: AH-AC, Glutinous Rice, Succinylation, Esterification

1. Introduction

Starch modification alters its natural properties and enhances its functional properties; it is achieved by applying heating and cooling cycles, chemical or enzymatic methods, or a combination of these [1,2]. The combination of autoclaving (high pressure) and heating (high temperature) will reduce the viscosity caused by changes in the starch granule structure and lower starch digestion rates [3,4]. Between the two methods, acid hydrolysis or heating-cooling methods are relatively more cost effective. Moreover, acid modification is a prevalent practice in the starch industry, in which starch is utilised in a multitude of applications, including in the manufacturing of food, paper and textiles, amongst other products [5]. The two modification processes, acid hydrolysis and hydroxypropylation, have no effect on granule size and paste formation properties, while significantly reducing the glass transition temperature [6].

Starch can be modified through esterification; that is, by replacing the hydroxyl groups in the starch molecules with organic acids. The process alters its molecular structure, rendering it unrecognisable to digestive enzymes [7] and modifying its polarity [8]. It also elevates the rheological properties of starch, thereby facilitating its use in the pharmaceutical domain as a vehicle for carrying bioactive components (a drug delivery system) [7], and in

emulsifiers, capsules, biofilm, edible coating and gel formers. In addition to organic acids, such as folic acid [7], citric acid and lactic acid [9], esterification can also be conducted using octenyl succinic anhydride (OSA) [10,11].

In Indonesia, an agricultural country, rice represents the primary staple food. People cultivate a variety of rice types, including the black glutinous, which is rich in amylopectin and anthocyanin, and can develop a viscous consistency in the cooking process. This unique characteristic could resulting specific modified starch that probably will impact the functional properties. Local Indonesian black glutinous rice contains 219.35 mg/100g anthocyanins, 13.32% amylose and 70.24% amylopectin [12], while black (purple) glutinous rice varieties in Thailand contain levels of anthocyanins ranging from 57–442 mg/100g and 3.4 – 9.7% of amylose [13]. Anthocyanins are known to be bioactive substances that are beneficial to human health; for example, controlling hypertension and type 2 diabetes [14], and in relation to cardiovascular and neurodegenerative diseases [15].

Additionally, black glutinous rice starch has the potential to be developed into novel foods due to its low digestibility, affordable price and insignificant impact on the taste or flavour of a product. Modifying the starch can yield the desired product characteristics while also providing added value; for example, reduced digestibility and altered pasting properties. However, its modification, especially with OSA esterification and AH-AC (acid hydrolysis-autoclaving cooling) methods, remains understudied. Current starch modifications through esterification using OSA mainly based on maize starch (54%), potato (10%), barley (5%), unspecified origins (17%), rice (8%) and 6% other origins [8].

The black glutinous rice used in this study was cultivated by local farmers in Central Java, Indonesia. The study aims to investigate the impact of modifying black glutinous rice starch using two different methods, AH-AC and esterification with OSA, which were chosen due to their cost effectiveness and scalability. The two methods are expected to provide modified starch products with the desired characteristics, presenting a promising avenue which offers the potential for diverse applications in the industry.

2. Materials and methods

2.1 Materials

The black glutinous rice starch used in this study was the result of isolation from black glutinous rice using the method of delivered of Mustofa et al. [12]. The starch was stored at room temperature (26-30°C) in a dark, shaded room until ready for use. The three chemicals used were hydrochloric acid (HCl), NaOH (Merck, Germany) and anhydrous octenyl succinate (Sigma-Aldrich).

2.2 Acid Hydrolysis Process

Acid hydrolysis and autoclaving-cooling processes were performed following the methodology proposed by Kusnandar et al. [16], with some modifications. 300 grams of black glutinous rice starch was combined with 1.2 litres of 1% HCl (1:4 w/v) and incubated at 35°C for 24 hours using a water bath shaker. Subsequently, the acid-treated starch slurry was neutralised with 1 M NaOH. The starch suspension was then stored in a refrigerator at 4°C for 24 hours, dried at 40°C, ground, and filtered using a 100-mesh sieve.

2.3 Autoclaving-Cooling Process

The resulting black glutinous rice starch (300 g) from the acid hydrolysis process, combined with 1.2 litres of distilled water (1:4 w/v), was placed in a glass beaker and subjected to an autoclaving-cooling cycle in an autoclave at a temperature of 121°C for 30 minutes. The autoclaved starch solution was then allowed to cool to room temperature and stored at 4°C for 24 hours. The autoclaving-cooling process was repeated three times, after which the solution was dried at 40°C, sieved, and filtered using a 100-mesh sieve.

2.4 Succinylation

The succinylation method employed chemical OSA, as proposed by No and Shin [10], with some modifications. 200 g of starch was dissolved in 450 mL of distilled water, with stirring. The pH of the solution was then adjusted to 8.5 using 2% NaOH. OSA, at a concentration of 3% relative to the dry starch, was added to the solution by stirring at room temperature. The pH of the solution was maintained at 8.5. After six hours, the pH was reduced to 7.0 using 5 M HCl. The solution was then subjected to centrifugation at 1400g for 15 minutes. Subsequently, the starch was washed twice with distilled water and on two other occasions with ethanol. Following filtration, the starch was dried at 40°C for 17 hours and sieved with a 100-mesh sieve.

2.5 Parameter Analysis

The modified starch was tested for moisture, crude fibre content and resistant starch using the method proposed by AOAC [17]. The amylose, amylopectin [18], anthocyanin [19], total phenolic content and 2,2-diphenyl-1-picrylhydrazyl (DPPH) [20], and water holding capacity (WHC) [21] of the starch were also assessed. Swelling power and pasting properties were determined using the method described by W. Zhang et al. [11]. The degree of substitution (DS) for OSA-modified starch was calculated using the No and Shin [10] method and formula. Scanning electron microscopy of the modified starch was performed according to the methodology described by [9].

2.6 Statistical Analysis

All analyses were repeated three times. Subsequently, the data were processed using one-way ANOVA to identify any significant difference between treatments. Duncan's multiple range test (DMRT) ($p < 0.05$) was used as a further analytical procedure if differences were found. The analysis process utilised international business machines (IBM) statistical package for the social sciences (SPSS) version 27.

3. Results and discussion

Modification altered the physicochemical characteristics and microscopic structure of the starch. In this research, the moisture content of the OSA- and AH-AC-modified starch was 5.22% and 7.83% respectively (Table 1). da Silva et al. [14] , who developed a simple extraction process without NaOH, produced modified starch with a notably disparate moisture content. The native black rice starch under research had a moisture content of 13.29%, ash content of 0.68%, fat content of 0.28%, protein content of 2.60%, carbohydrate content of 83.15%, and an amylose content of 20.02%. In addition, Mustofa et al. [12] reported on native black glutinous rice starch with 9.08% moisture, 2.41% ash, 0.11% fat and 1.44% protein. Gani et al. [22] observed that the starch isolation method influenced the nature and pasting properties of the resulting starch.

Table 1 Chemical properties of modified black glutinous rice starch.

Parameter	Native starch*	AH-AC	OSA
Moisture content	9.08±0.49^{c}	7.83±0.04^{b}	5.22±0.03^{a}
Crude fibre (% db)	0.00±0.00^{a}	0.20±0.02^{b}	0.45±0.02^{c}
Starch (% db)	83.02±0.43^{a}	88.07±0.27^{b}	83.11±0.23^{a}
Amylose (% db)	17.30±0.30^{c}	7.94±0.01^{b}	7.43±0.06^{a}
Amylopectin (% db)	65.72±0.25^{a}	80.13±0.27^{c}	75.67±0.23^{b}
Resistant Starch (% db)	0.62±0.00^{a}	9.27±0.04^{b}	10.38±0.02^{c}
Anthocyanin (mg/100g)	16.02±0.22^{c}	1.09±0.00^{a}	5.41±0.03^{b}
Total Phenolic Content (mg GAE/g)	0.03±0.00^{a}	132.36±1.74^{b}	153.39±1.90^{c}
Antioxidant Activity DPPH (%)	22.89±0.15^{b}	14.06±0.08^{a}	38.85±0.08^{c}
Water Holding Capacity (%)	107.55±0.38^{a}	111.95±0.49^{b}	113.30±0.64^{c}
Swelling Power (%)	2982.37±21.91^{c}	150.12±2.60^{a}	1235.89±2.81^{b}
Degree of Substitution (DS)		(none)	0.26±0.01
Pasting Properties:			
Peak Viscosity (cP)	5001.00±0.00^{c}	60.00±0.00^{a}	434.00±0.00^{b}
Trough Viscosity (cP)	1522.00±0.00^{c}	41.00±0.00^{a}	432.00±0.00^{b}
Break Down (cP)	3479.00±0.00^{c}	19.00±0.00^{b}	2.00±0.00^{a}
Final Viscosity (cP)	2933.00±0.00^{c}	57.00±0.00^{a}	608.00±0.00^{b}
Set Back (cP)	1471.00±0.00^{c}	16.00±0.00^{a}	176.00±0.00^{b}
Peak Time (Min)	7.93±0.00^{b}	4.27±0.00^{a}	9.80±0.00^{c}
Pasting Temperature (°C)	80.90±0.00^{a}	nd	81.35±0.00^{b}

* Mustofa et al. [12]

Its capacity to retain water was contingent upon the presence of other macromolecular components and the intrinsic properties of the starch. The macromolecules identified in the modified starch were fibre and starch, both having water-retaining capacity. This therefore signified that starch modification was an effective method for escalating its fibre content. Similar result were presented in research on the modification of jack bean (*Canavalia ensiformis*) starch using the autoclaving-cooling method [21]. Nonetheless, this phenomenon is not universal, as

suggested by T. Li et al. [3], who reported that the crude fibre content of purple sweet potato starch decreased after modification using acid hydrolysis and autoclaving-cooling methods.

The starch content in AH-AC-modified starch was higher than in the OSA-modified form, while OSA-modified starch was higher in fibre content. Native black glutinous rice starch exhibited a composition of 17.30% amylose and 65.72% amylopectin [12], whereas the modified starch displayed a reduction in amylose and an increase in amylopectin. AH-AC modification causes a decrease in amylose content and an increase in amylopectin content. In this study, the HCl level used was 1%, but it had the same impact as in Kusnandar et al.'s [16] research, which used 2% HCl with AH-AC treatment. It can therefore be assumed that the amylose of black glutinous rice starch is more susceptible to degradation during the acid hydrolysis process but is not accompanied by degradation of amylopectin. In a separate study, Faridah et al. [4] found that several treatments, namely acid hydrolysis, autoclaving-cooling and a combination of both, could reduce amylopectin levels and increase those of amylose in arrowroot starch. Modification with OSA also causes a decrease in amylose and an increase in amylopectin. This is thought to be due to the presence of ester bonds between OSA and hydroxyl groups in the amylose chain, causing changes in the structure of amylose, which prevent the formation of helical structures that are characteristic of amylose [23]. The ester bond also prevents the absorption of iodine used to measure amylose content [20].

Furthermore, the resistant starch of AH-AC- and OSA-modified starch increased. Similar results have been reported on the use of a dual modification between acid hydrolysis and heat moisture treatment (HMT) [1]. Glutinous rice (waxy rice), with a high amylopectin content, has a tiny granular size in comparison to other types of starch, including corn, potato and wheat starch. This allows the acid to penetrate the starch more efficiently, thereby facilitating complete hydrolysis. Li and Hu [24] reported that amylose chains with a degree of polymerization (DP) exceeding ~300 and long inter-cluster chains of amylopectin undergo a rapid degradation phase during acid hydrolysis. Conversely, amylose chains with a DP below ~300 are likely to experience a slower degradation.

Acid hydrolysis was performed below the gelatinisation temperature. The rate of acid hydrolysis goes in line with the length of time of the hydrolysis treatment [6]. In consequence, amylose levels would increased subsequent to modification, but amylopectin levels decreased following the AH-AC process. This was corroborated by a study conducted by Hung et al. [1], who suggested that during the acid hydrolysis process, amylopectin chains were cut into straight-chain molecules, for example amylose. However, in contrast to the cutting by more specific enzymes, acid cutting occurred in amylopectin and short-chain amylose [1].

Reduced amylose content in OSA-modified starch demonstrates that the amylose structure was subjected to attack by the aryl groups present in OSA. Borah et al. [7] demonstrated that starch was inherently structured in a granule form and exhibited partial crystallinity. The amylose component is situated within the amorphous region of the granule. The esterification of starch entailed the removal of three available -OH groups and their replacement with alkyl groups or aryl derivatives. The esterification of starch with high crystallinity is an arduous process, with the degree of substitution (DS) of the resulting product being inversely proportional to the initial starch crystallinity. The DS was directly proportional to the amylose content of the rice starch studied and indicated the extent to which a hydroxyl group can be replaced by an aryl group. Based on the number of esterified groups, the degree of substitution of esterified starch has been categorised into three groups: low (0.01-0.2), medium (0.2-1.5) and high (1.5-3.0) [25]. The degree of substitution of black glutinous rice starch modified with OSA was 0.26, therefore in the low category.

The attachment of acyl or aryl groups increased starch polarity, which in turn impacted the solubility of the modified starch in water. Improving water solubility or binding bioactive components based on their polarity also necessitatesd the esterification process. In general, the higher the amylose content of the starch, the higher the DS of its modified form. This phenomenon was observed in a study conducted by Zhang et al. [11], who modified rice starch using OSA. Their outcome highlighted that more hydroxyl groups were susceptible to attack in the presence of OSA and the resulting esterification.

As the degree of esterification increased, the solubility of starch also rose. The attachment of aryl groups increases the polarity of the starch molecule, thus promoting solubility. Additionally, the esterified form of starch showed enhanced emulsifying capabilities [26]. This property contributed to preparing starch as a carrier for bioactive compounds, which are typically non-polar. According to Ačkar et al. [2], the attachment of succinate, an organic acid, to starch molecules offers several benefits, including enhanced solubility in cold water, high viscosity, effective thickening, increased clarity of the paste, prevention of breakage, and good stability during the freeze-thaw process. The characteristics of acetylated starch differed slightly from those of the succinylated one. The paste produced from acetylated starch was less clear, the gel was less viscous, and the gelatinization temperature was lower.

The starch modified with OSA demonstrated an enhanced capacity to absorb water in comparison to the native form [12]. Native starch had a water holding capacity (WHC) of 107.55%, while that of AH-AC- and OSA-modified starches were 111.95% and 113.30% respectively. This aligns with the findings of Lase et al. [27], who demonstrated that sweet potato starch modified using the HMT method showed an augmented capacity for water

absorption, as evidenced by an elevated water absorption index. The greater the DS, the greater the ability of the esterified starch to absorb water and oil.

It has been proposed that starch esterification using OSA increases the amphiphilic properties of the resulting starch [8]. Nevertheless, according to Masina et al. [28], esterification with aryl groups causes neither an increase in the swelling ability of the starch, nor in its swelling properties. In other words, the swelling power of modified starch is significantly lower than that of the unmodified form.

The combination of the hydrolysis process using acid and the heat and cold cycle had a significant effect on the integrity of the starch granule. This led to a notable decline in swelling power, with a reduction from 2,982.37% [12] to 150.12%. The swelling power of OSA-modified starch was higher relative to that of the AH-AC-modified form, with a slight decrease compared to that of the native starch [12]. Adding high-swelling power starch could elevate the texture and volume of baked goods, such as bread, cakes and pastries, by facilitating the retention of moisture, which generates softer crumbs. Moreover, its addition to dairy products could enhance their thickness and stability, providing a creamy texture, while in the case of sauces, soups, frozen foods and meat products, high-swelling power starch could serve as a suitable fat replacer. The active groups present in OSA-modified starch could be attached to other active groups, allowing for a more extensive range of applications; for example, as a vehicle for drugs or other bioactive compounds.

As previously mentioned, the resistant starch content was higher in the OSA-modified than in the AH-AC-modified starch. The esterification of starch renders it unrecognisable to digestive enzymes. Furthermore, esterification enhances slowly digested starch (SDS) levels [8], as documented in the study by W. Zhang et al. [11], who found that the resistant starch content of Japonica rice starch increased from 4.87% to 25.30% following esterification. Goñi et al. [29] demonstrated that food containing resistant starch levels ranging from 5-15% were high-resistant-starch types.

Modification with OSA generated starch with 5.41 mg/100g anthocyanin, antioxidant activity of 38.85%, and total phenolic content (TPC) of 153.39 mg GAE/g, while the anthocyanin, antioxidant activity and TPC contents of native starch were 16.02 mg/100g, 22.89% and 0.03 mg GAE/g respectively. The reduction in anthocyanin and antioxidant activity in starch modified with OSA was less significant than that in that modified with AH-AC (Table 1).

Kanha and Laokuldilok [30] found a reduction in anthocyanins in black glutinous rice bran during the drying process. Similarly, processing black glutinous rice starch using esterification OSA and AH-AC methods needed a drying step. Starch modified by the AH-AC method can undergo three cycles of cooling and heating (autoclaving), suggesting that the heating process experienced by the starch is three times that of the OSA-modified type. Consequently, the levels of anthocyanins, antioxidant activity and TPC of this starch were higher than those of the OSA-modified form. High temperature or acidity will greatly influence the stability of anthocyanins [31,32].

Furthermore, the antioxidant activity of OSA-modified starch was found to be superior to that of the native type, as was also the case with total phenol. Regrettably, research on the impact of esterification using OSA on coloured rice starch has yet to be conducted.

The pasting temperature for AH-AC-modified starch was unsuccessfully quantified (Table 1). Figure 1 offers a more detailed illustration of such pasting properties, demonstrating that the alteration in viscosity was consistent with the increase in temperature and the shear stress applied, although no significant variation was observed. The application of a temperature range of 55-65°C caused no significant alteration in viscosity. As also illustrated in Figure 1, the viscosity of the gel exhibited a tendency towards a stable form. The peak viscosity (PV) of AH-AC-modified starch was classified as very low (60 cP) in comparison to that of the OSA-modified form (Figure 1).

An examination of the pasting properties of the two modified starches revealed that the OSA-modified type possessed certain properties offering advantages to processing technology, namely stability during heating and the application of shear stress. Ghalambor et al. [6] report that starch modification via acid hydrolysis in conjunction with hydroxypropylation will lead to a reduction in the pasting temperature. In this study, no discernible change in the gelling temperature was observed. Ghalambor et al. [6] also suggest that acid hydrolysis treatment of sago starch does not alter the size of the starch particles.

Applying heat to a starch solution at 50°C produced swelling of the amylose present within the starch granules, subsequently destroying the crystalline structure of the amylopectin, resulting in the bursting of the granules. When the starch was then cooled, a retrogradation process occurred rapidly in the amylose due to cross-linking in hydrogen bonds, but slowly in the amylopectin due to its branching structure [6].

Scanning electron microscopy of the two modified starches indicated alterations in their structures. A previous study [12] highlighted that the structure of native black glutinous rice starch was polygonal, with a size of 2-5 μm. In this study, the starch modified using AH-AC showed an irregular shape, lacked corners, and displayed a tendency to form plates with an uneven, wavy surface.

Additionally, the dimensions of the starch granules underwent alterations in length, width and thickness (Figure 2). In contrast, the size of sago starch granules yielded through modification by acid hydrolysis combined with hydroxypropylation remained unaltered [6].

(A)

	0,07	0,13	0,20	0,27	0,33	0,40	0,47	0,53	0,60	0,67	0,73	0,80	0,87	0,93	1,00	1,07	1,13	1,20	1,27	1,33	1,40	1,47	1,53	1,60	1,67	1,73	1,80	1,87	1,93	2,00	2,07	2,13	2,20	2,27	2,33	2,40	2,47	2,53	2,60	2,67	2,73	2,80	2,87	2,93	3,00	3,07	3,13	3,20	3,27	3,33
A Viscosity	0	162	140	140	17	39	42	40	40	42	42	40	41	43	42	39	42	45	42	41	41	42	44	41	43	42	43	41	42	43	42	43	43	42	43	44	44	43	45	44	45	46	46	47	48	47	48	49	50	50
A Temperature	48,30	48,25	49,20	49,85	50,05	50,15	50,20	50,30	50,35	50,35	50,30	50,30	50,30	50,25	50,25	50,20	50,20	50,40	50,80	51,15	51,50	51,90	52,35	52,75	53,15	53,60	54,05	54,40	54,75	55,20	55,55	56,00	56,30	56,85	57,15	57,55	57,95	58,25	58,65	59,05	59,40	59,75	60,15	60,50	60,85	61,25	61,35	62,00	62,35	62,70
A Speed	953	960	340	159	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	150	160	160	160

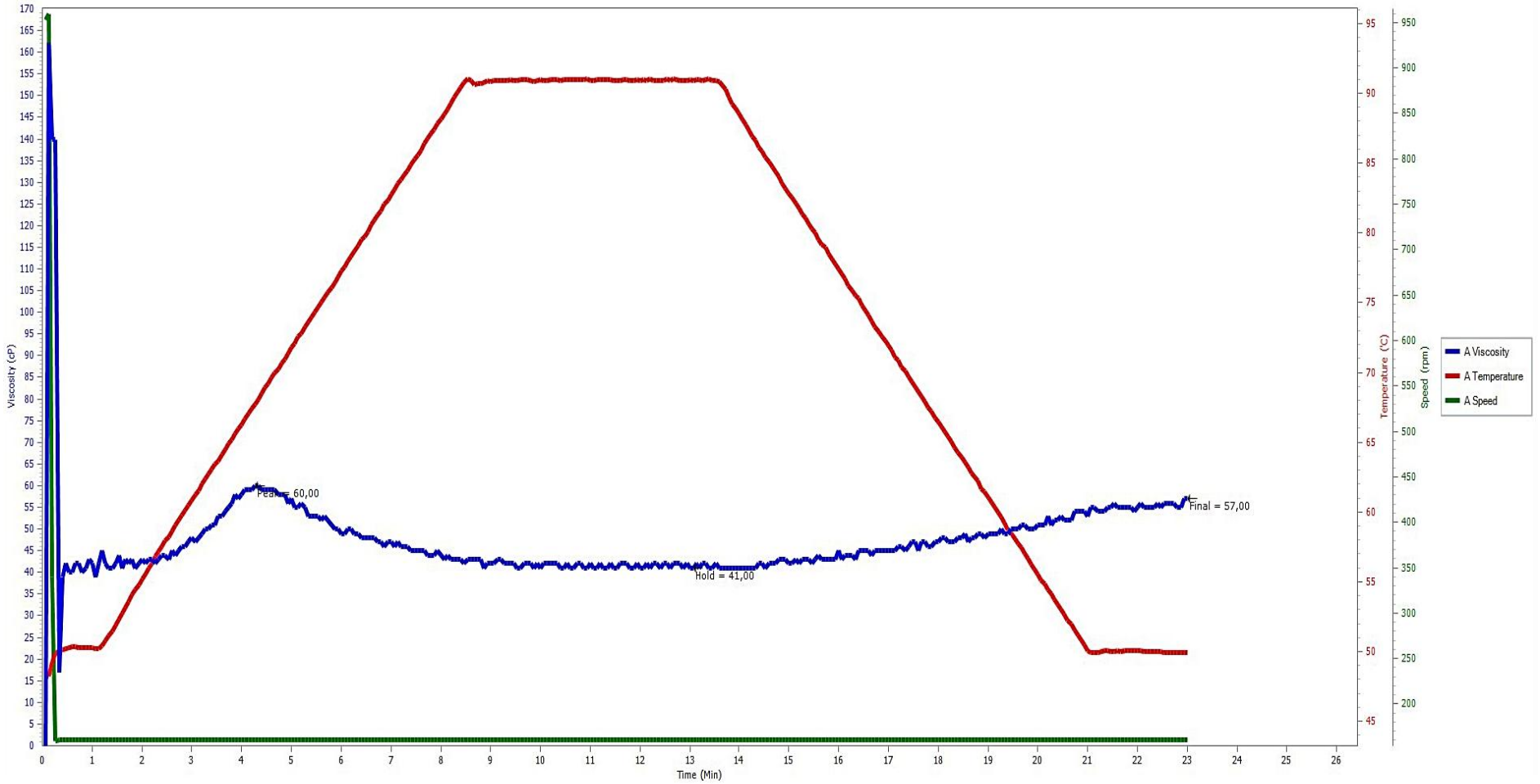

Figure 1 Pasting properties of modified starch: AH-AC (A) and OSA (B).

(B)

	0,07	0,13	0,20	0,27	0,33	0,40	0,47	0,53	0,60	0,67	0,73	0,80	0,87	0,93	1,00	1,07	1,13	1,20	1,27	1,33	1,40	1,47	1,53	1,60	1,67	1,73	1,80	1,87	1,93	2,00	2,07	2,13	2,20	2,27	2,33	2,40	2,47	2,53	2,60	2,67	2,73	2,80	2,87	2,93	3,00	3,07	3,13	3,20	3,27	3,33
B Viscosity	0	110	110	-117	22	14	16	14	15	14	16	15	15	15	16	16	15	16	16	15	15	15	15	16	14	15	14	14	16	15	15	15	15	15	15	15	14	15	15	16	14	14	15	14	14	16	15	15	15	14
B Temperature	47,95	47,95	48,95	49,90	50,15	50,25	50,35	50,40	50,40	50,40	50,40	50,40	50,40	50,35	50,30	50,20	50,25	50,50	50,80	51,20	51,60	51,90	52,40	52,80	53,25	53,60	54,00	54,40	54,80	55,20	55,55	56,05	56,30	56,75	57,05	57,55	58,00	58,25	58,70	59,05	59,40	59,80	60,10	60,45	60,80	61,20	61,55	61,95	62,30	62,70
B Speed	948	960	665	139	164	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	160	150	160	160	160

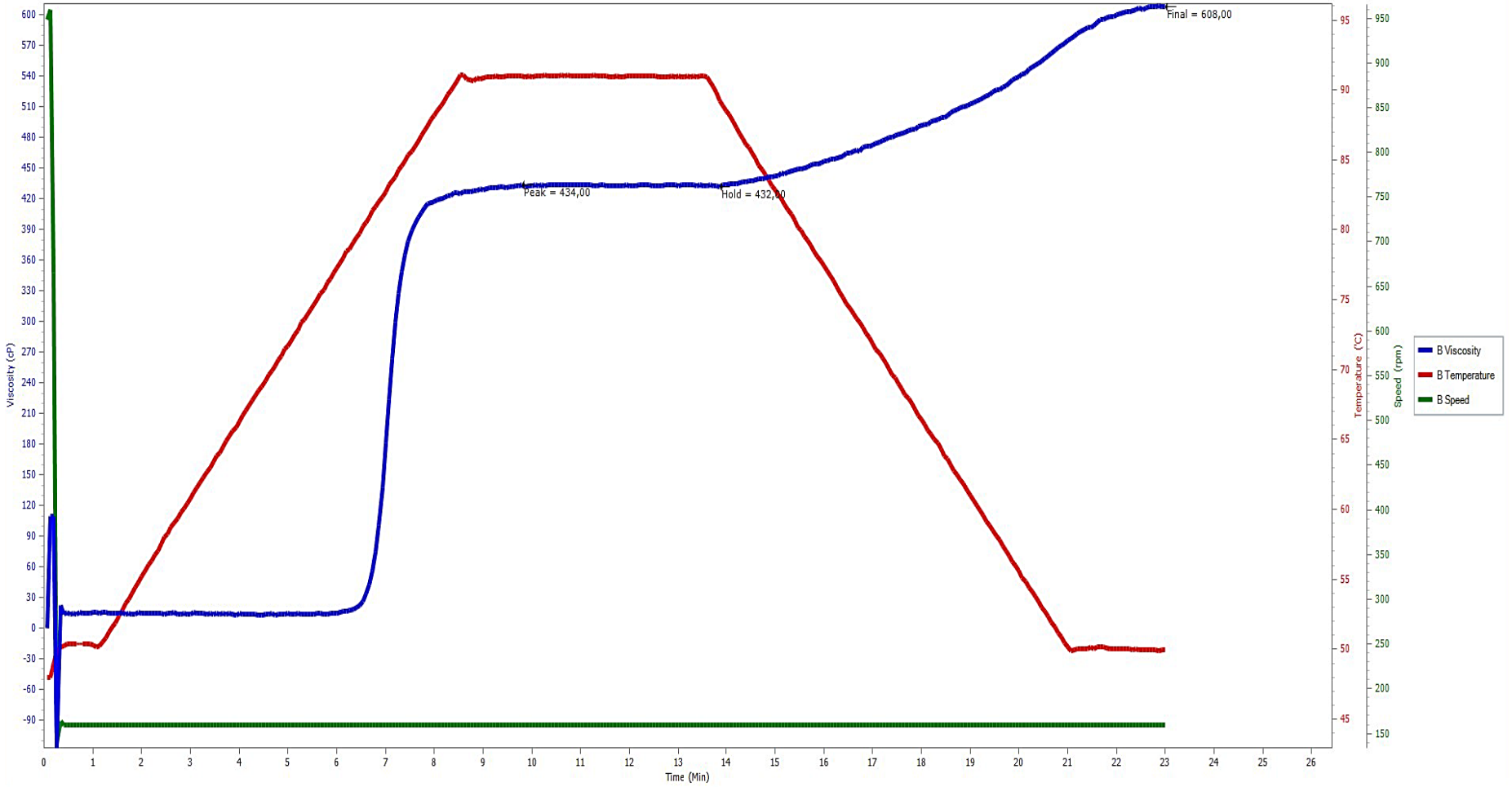

Figure 1 (cont.) Pasting properties of modified starch: AH-AC (A) and OSA (B).

It was also observed that the starch granules in this study could be cut into smaller, thinner ones. The combined treatment of acid hydrolysis and autoclaving-cooling had a more pronounced impact on the pasting properties of the starch than they had in isolation, as acid hydrolysis increased the number of short chains formed from amylose and amylopectin [24]. The autoclaving-cooling process facilitated gelatinisation and retrogradation processes, leading to the cutting of more amylose or amylopectin chains, which then took place in the retrogradation process to form resistant starch [4].

OSA-modified starch had a lower peak viscosity relative to the native type [12], at 434 cP < 5001 cP. High interaction between esterified starch and water resulted in intermolecular resistance, thereby reducing viscosity and inter-mass resistance. Similarly, the setback viscosity was found to decrease after succinylation. However, a different finding was documented in a separate investigation by No and Shin [10], who found that the setback viscosity of esterified rice starch increased when the OSA-modified form was administered at a concentration of 3%. Conversely, the administration of OSA-modified starch at concentrations of 1 and 2% caused setback viscosity to decline. The extent of succinylation, which was affected by the concentration of OSA-modified starch given, had a significant impact on the viscosity profile. As the concentration of OSA-modified starch was raised, the DS also increased.

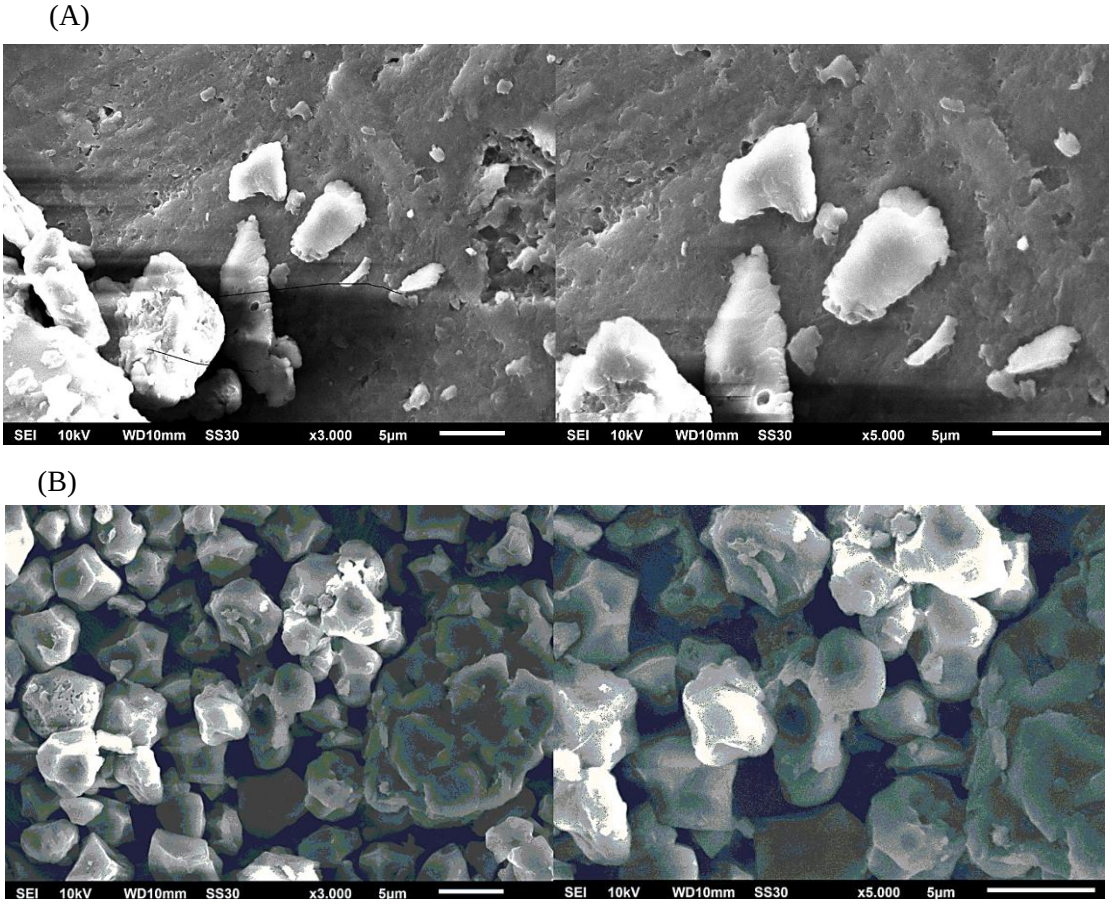

Figure 2 Starch granule: AH-AC (A) and OSA (B)

The modified starch amylograph (Figure 2) differed in starch structure from the native type [12], with the surfaces becoming bumpy and porous, in line with Liang et al. [33]. Marta et al. [34] reported that more structural changes occurred in starches treated with high osmotic pressure, cold heat cycling, and the addition of citric acid, followed by OSA application. However, it should be noted that the esterification of starch using OSA has a purpose besides improving its resistance, namely increasing its amphiphilic properties.

Finally, the microscopic structure of the starch granules underwent significant alterations. The native starch demonstrated a smooth and polygonal surface, with a visible angle between one surface and another [12]. In contrast, the modified starches, both OSA- and AH-AC-modified forms, showed a rough and porous surface structure, with the polygonal structure being less visible, especially in the AH-AC-modified version. These results differ from those observed in rice and potato starch modification by Martins et al. [5] and in rice starch modification by Butt et al. [9] using acid hydrolysis. The discrepancy in the results can be attributed to the

prolonged duration of acid soaking employed in this study, which was 24 hours (35°C, HCl), whereas Butt et al. [9] and Martins et al. [5] performed the process for 12 hours (25°C, lactic acid/citric acid) and 5-8 hours (50°C, HCl/citric acid) respectively. In addition to duration differences, the types of acid and temperature employed were also different. M. Zhang et al. [35] concurred that different types of organic acid, duration and retrogradation temperature influenced the physicochemical properties of acid hydrolysis starch. Furthermore, increasing acid hydrolysis time reduced swelling properties (Table 1) and syneresis possibility (Figure 2). The retrogradation rate of acid-treated starch gels further increased with increasing hydrolysis.

Considering the pasting properties of AH-AC and OSA starch, the former is difficult to gel and has a low viscosity, making it suitable as a topping for products that do not require high viscosity. It can also be used as a filler in candies that do not require high viscosity; as a coating with a light texture on fried foods; and as a low-calorie product, and is also beneficial due to its high resistance starch, but lack of stickiness due to its low viscosity. In different conditions, OSA starch is suitable as an emulsifier because of its amphiphilic nature; it can also be used for products that need viscosity but quickly form solids, such as puddings, and is suitable for sauces that require starch with stable viscosity. It can also be used for products that need to be soft and juicy after cooking.

4. Conclusions

Modifying black glutinous rice starch significantly altered its pasting behaviour and physicochemical characteristics, especially its gel stability in response to temperature change. Modification with OSA enhanced the resistant starch content, which was higher than the AH-AC-modified type. High heating and acid treatment, as used in AH-AC modification, greatly affected the anthocyanin content and antioxidant activity of the starch compared to treatment with OSA. The pasting properties of OSA- and AH-AC-modified starches were different from those of native starch. OSA-modified starch demonstrated higher PV and trough viscosity (TV) relative to the AH-AC-modified form but exhibited a relatively low DS. Despite a notable reduction in viscosity, the pasting temperature remained unaltered following succinylation. Additionally, the morphology of the starch underwent alterations in terms of shape, which directly impacted its potential applications.

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