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Research article

Formation of Type III Resistant Starch and Alterations in Starch Characteristics and Pasting Behavior of Arrowroot Starch by Autoclaving-Cooling Treatment

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Abstract

Resistant starch (RS) is employed as a functional food component to prevent non-infectious diseases. Due to its generous amylose content, which ranges from 20.5% to 30.27%, arrowroot tuber is a potential source of RS. The RS content of tuber starch can be further enhanced through physical modifications, such as an autoclaving-cooling cycle to generate type III RS. This research aimed to determine the optimal autoclaving temperature and duration in order to produce arrowroot starch with the maximum RS content, as well as to characterize its properties. The arrowroot starch was autoclaved at temperatures of 105°C, 120°C, and 135°C for 20, 40, and 60 min, respectively. The autoclaving cooling modification was carried out over 5 cycles. Based on the findings, the arrowroot starch with the highest RS content (5.02%) was generated by autoclaving at 105°C for 40 min. This was higher than that of the native starch (1.24%). This modified starch with the highest RS comprised 10.49% moisture content, 86.75% starch content, 27.93% amylose content, and 58.82% amylopectin content. The modified starch had higher amylose content than native starch (25.43%). Additionally, it had a bulk density of 0.68 g/mL, 2.24 g/g swelling power, 1.82% solubility, 0.83 g/g water absorption, and 83.33 brightness value. The swelling power and the solubility of modified starch were lower than the native starch. Amylograph curves demonstrated that the autoclaving-cooling modification generated starch with a more stable pasting profile. These results show that the autoclaving-cooling method improved the amylose and RS content of the modified starch. However, the modified starch has a lower value of pasting properties. Lower values

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for these viscosity parameters may be desirable in a variety of food applications that require a less viscous, more stable, and less retrograded starch paste.

Keywords: arrowroot tuber; autoclaving-cooling modification; pasting behavior; resistant starch; starch properties

1. Introduction

Non-infectious diseases, such as cancer, diabetes, cardiovascular disease, stroke, and kidney, and lung disease, are not spread from one person to another. Environmental and lifestyle changes are factors that influence disease patterns in Indonesia, which are dominated by non-infectious diseases (Purnamasari, 2018). Non-infectious diseases can be mitigated with the implementation of an appropriate diet and physical activity. These lead to in a transition in dietary preferences towards functional foods (Greenberg & Deckelbaum, 2016). Functional foods can offer physiological advantages or diminish the risk of non-infectious diseases. Ingredient that is frequently employed in the food industry is resistant starch (RS). RS and its derivatives are nondigestible in the digestive tract. Crops such as grains, tubers, pulses, and legumes are good sources of RS (Fuentes-Zaragoza et al., 2010; Nigudkar, 2014).

Tubers possess the capability to serve as a source of RS because they contain higher natural RS than cereals or other sources (Chen et al., 2010; Arifani & Tamalea, 2024; Makiyah et al., 2024; Gunawan et al., 2025). Arrowroot tuber is a type of tuber that is still rarely utilized, even though it has a high natural RS content. The RS percentage in arrowroot starch varies from 1.85% to 3.50% (Sugiyono et al., 2009). Numerous methods exist for enhancing the RS content of tuber starch, including chemical, enzymatic, physical (Ardhiyanti et al., 2017), and fermentation (Magfiroh et al., 2025) modifications. Starch is frequently modified physically since it is a simpler method and leaves no possible chemical reagent behind (Ardhiyanti et al., 2017). Furthermore, physically modified RS (type III RS) is relatively heat-resistant, which enables it to improve the preservation of its properties during processing (Sugiyono et al., 2009; Song et al., 2012). The process of physically modifying starch often employs starch with a high amylose concentration, as amylose is more susceptible to retrogradation than amylopectin (Herawati, 2011). Amylose content in arrowroot starch is higher than that of other tuber starches, ranging from 20.5 to 30.27% (Purba, 2007; Faridah et al., 2014; Maulani & Hidayat, 2016).

Hydrothermal technology, a commonly utilized method for starch modification, employs moisture and heat to alter the characteristics of starch (Krisna, 2011; Yovani et al., 2022; Bimrew et al., 2025). Autoclaving-cooling cycles represent a hydrothermal method that can substantially enhance RS concentration. Numerous investigations have demonstrated that, in comparison to one cycle modified starch, increasing the number of autoclaving-cooling cycles can raise the concentration of RS. When maize flour was modified using two autoclaving cooling cycles, the concentration of RS increased from 15.84 to 27.78% while the starch digestibility decreased from 67.02 to 35.74%. Moreover, two cycles of autoclaving-cooling treatment also resulted in an increase in the concentration of RS in maize starch. While starch digestibility decreased from 76.15 to 28.09%, RS increased from 15.27 to 32.53% (Faridah et al., 2022). Additionally, RS concentration increased in Foxtail Millet Starch treated with two autoclaving-cooling cycles as opposed to one cycle (Surawan et al., 2024). The autoclaving-cooling cycles elevated the value of RS in arrowroot starch from 2.12% to 12.15% after 5 cycles (Sugiyono et al., 2009), and in taro starch from 1.25% to 4.38% after 2 cycles (Wiadnyani et al., 2015).

Increased amylose breakdown from more autoclaving-cooling cycles can produce a short-chain starch fraction, which influences the RS generation (Zheng et al., 2023). The efficacy of RS generation via the autoclaving-cooling cycle of arrowroot starch is contingent upon various parameters, including the number of cycles, autoclaving duration and temperature, in addition to cooling duration and temperature (Rosidah, 2014; Lilia-Baby et al., 2016). Research findings regarding the manufacture of resistant starch from arrowroot tubers by physical modification techniques are scarce, indicating a significant urgency for further investigation. The aim of this research is to identify the temperature and autoclaving time that yielded the highest RS content in arrowroot starch, as well as to characterize the properties of the modified arrowroot starch with the highest RS content. The study may advocate for the utilization of arrowroot tubers as a regional food source and provide a healthier option for customers owing to their elevated RS concentration.

2. Materials and Methods

2.1 Materials

Arrowroot tubers (10-month-old) (*Maranta arundinacea* L.) from Bojonegoro, East Java, distilled water, α-amylase (#Merck A3306), amyloglucosidase/AMG (#Merck A7420), 99% ethanol (#Merck 459844), 95% ethanol (#Merck 493511), KOH (#Merck 484016), glucose oxidase peroxidase reagent (#Merck G3660), Whatman quantitative filter paper (Grade 41) (#Merck WHA1441125), and all pro analysis reagents such as NaOH, CH_3COOH, H_2SO_4, HCl, I_2, and KI, were used in this study.

2.2 Methods

2.2.1 Arrowroot starch extraction

The extraction of starch followed Mariati's procedure (Mariati, 2001). The arrowroot tubers were initially peeled to remove the skin, dirt, and adhering roots. The tubers were then soaked for approximately 1 h to soften them, facilitating the grating and washing processes. Subsequently, grating was performed to disrupt the cells and tissues of the tuber, which aided in the extraction of starch with the addition of water at 1:3.5 (w/v) tuber-to-water ratio. The extraction technique was executed twice by reusing the arrowroot pulp. The starch solution was allowed to precipitate for 12 h and then dried (50°C; 6 h). The desiccated starch was pulverized and sieved through a 60-mesh screen.

2.2.2 Autoclaving-cooling for arrowroot starch modification

The approach for altering arrowroot starch using the autoclaving-cooling cycle method is based on Rosidah (2014). The first step involved dissolving arrowroot starch in 20% (w/v) distilled water and heating the mixture to 70°C while stirring until homogeneous. The starch underwent heating in an autoclave for durations of 20, 40, and 60 min at temperatures of 105, 120, and 135°C, respectively. Following the heating process, the starch was permitted to cool at room temperature for about 1 h to prevent additional gelatinization. The starch was thereafter stored (4°C; 24 h). The heating and cooling procedure was performed four times, yielding an overall of five autoclaving-cooling cycles. Next, the starch was dried using an oven dryer at a temperature of 50°C for 6 h, then crushed and sieved (60-mesh).

2.3 Analysis procedures

2.3.1 RS content analysis

This technique adheres to the principles established by McCleary and Monaghan (2002). The non-RS was dissolved and hydrolyzed using amyloglucosidase (AMG) and α-amylase (37°C; 16 h). The hydrolysis process was then completed (enzyme inactivation) with the addition of 99% ethanol. After inactivation, the sample was subject to centrifugation, and the resulting pellet (RS) was collected and rinsed twice with 50% ethanol to eliminate glucose from the hydrolysate. The next step was to dissolve the RS by immersing the cleaned pellet in 2 M KOH for 20 min in ice water bath. The RS solution was subsequently neutralized with acetate buffer and hydrolyzed through to glucose using AMG (50°C: 30 min). The last stage involved measuring glucose derived from RS hydrolysis using the glucose oxidase-peroxidase (GOPOD) reagent. The RS level in the sample was determined by multiplying the observed amount of glucose by 0.9.

2.3.2 Color analysis

Color L* testing was conducted using the CIE Lab Color Space to obtain the a*, b* and L* values (Nadir et al., 2015). L* values range from 0 (dark) to 100 (bright). The a* value denotes the chromatic color of the green-red mixture with a value of -a* for green, and +a* for red. The b* value denotes the chromatic color of blue-yellow mixtures with a value of -b* for blue, and +b* for yellow. Color measurements were taken at three points on each starch sample. ΔE* was calculated using native starch as the standard, with equation 1.

$$\Delta E^{*} = \sqrt{(\Delta L^{*})^2 + (\Delta a^{*})^2 + (\Delta b^{*})^2} \quad (1)$$

Here, ΔE* represents the overall color difference value. ΔL* represents the disparity in lightness/darkness values (L*sample - L*standard). Δa* represents the disparity along the red/green axis (a*sample - a*standard). The Δb value represents the difference along the yellow/blue axis (b*sample - b*standard).

2.3.3 Bulk density analysis

The samples of starch were evaluated for bulk density using the methodology established by Alam et al. (2024). A precise volume measurement was obtained by placing 1 g of the sample into a 10 mL graduated cylinder. Five occurrences of moderate shaking and tapping were utilized to compact the starch surface. The bulk density was determined using equation 2.

$$\text{Bulk density (g/mL)} = \frac{\text{sample mass (g)}}{\text{sample volume occupied (mL)}} \quad (2)$$

2.3.4 Moisture and ash content analysis

Starch moisture content was measured based on the thermogravimetric methods (Halim et al., 2023)The starch sample was quantified to the nearest 1-2 g in a pre-weighed receptacle. It was then dried in an oven at 110°C for 3 h, cooled in a desiccator, and weighed until a stable weight was attained. The ash content of starch was ascertained by

the procedure outlined by Yovani (2022) through the drying ash method using an electric furnace (550°C).

2.3.5 Analysis of starch content

The starch content was quantified with the direct acid hydrolysis technique (Nielsen, 2010). The starch sample was initially rinsed with distilled water to eliminate soluble carbohydrates. The material was subsequently hydrolyzed using 25% HCl for a duration of 2.5 h. Following the hydrolysis process, the samples were neutralized using 1 N NaOH and subsequently filtered. The filtrate was analyzed for glucose via the decreasing sugar determination technique. The quantified glucose content was transformed into starch content through multiplication of the glucose content by 0.9.

2.3.6 Analysis of amylose and amylopectin content

Amylose content was measured using the iodine binding method (Juliano, 1971). Approximately 100 mg of starch sample was placed into a test tube, followed by the addition of 1 mL of 95% ethanol and 9 mL of 1 N NaOH. The test tube was heated in boiling water for approximately 10 min until a gel formed. Upon cooling, the solution was meticulously transferred into a 100 mL volumetric flask and adjusted to the calibration mark with water. Five mL of the solution were transferred into a 100 mL volumetric flask, thereafter, supplemented with 1 mL of 1 N acetic acid and 2 mL of iodine solution (0.2% I_2 in 2% KI), with water added to the calibration line. The solution was allowed to stand for 20 min prior to measuring the absorbance with a spectrophotometer at 625 nm. Using the formula obtained from the standard curve, the amount of amylose in the sample was ascertained. From the total starch content, the amylopectin content was calculated by subtracting the amylose content.

2.3.7 Analysis of crude fiber concentration

The content of crude fiber was evaluated utilizing the acid hydrolysis technique (Madhu et al., 2017). Two grams of the sample were dissolved in 1.25% H_2SO_4 (50 mL) and agitated (30 min) using a magnetic stirrer. The mixture was subsequently strained through muslin cloth and washed with boiling water until a neutral filtrate was achieved. Afterwards, 3.25% NaOH (50 mL) was introduced and heated to boiling (30 min). The sample was strained using muslin cloth again and rinsed with 25 mL of 1.25% boiling H_2SO_4 and thereafter subjected to three separate rinses with 50 mL of heated water and ultimately with 96% ethanol. The remaining substance was terminated and placed into a pre-weighed ashing dish (W1). Subsequently, it was dehydrated (130°C; 2 h). The sample was chilled in a desiccator, and its weight (W2) was documented. The residue was burned (600°C; 30 min), subsequently cooled in a desiccator, and then reweighed (W3). The sample's crude fiber content was calculated using equation 3.

$$\text{Crude Fiber Content (\%)} = \frac{(W2-W1)\,(W3-W1)}{\text{weight of the sample (g)}} \times 100 \quad (3)$$

2.3.8 Swelling power and solubility analysis

A starch mixture was produced by dissolving starch in distilled water (2%; w/v) (Wang et al., 2010). The suspension was boiled in a water bath with intermittent stirring (90°C; 30

min). The suspension was then subjected to centrifugation at 4000 rpm (15 min). The supernatant was removed from the precipitate (pellet), and the precipitate was subsequently weighed. The removed supernatant was decanted into a pre-weighed dish and subjected to drying in an oven (105°C) until a stable weight was attained. The solubility and swelling capacity of the sample were assessed using equations 4 and 5.

$$\text{Swelling power (g/g)} = \frac{\text{Weight of dish and precipitate (g)} - \text{Weight of empty dish (g)}}{\text{weight of the sample (g)}} \times 100 \quad (4)$$

$$\text{Solubility (\%)} = \frac{\text{Weight of dish and supernatant (g)} - \text{Weight of empty dish(g)}}{\text{weight of the sample (g)}} \times 100 \quad (5)$$

2.3.9 Water absorption capacity

The water absorption capacity was assessed according to the methodology described by Sathe and Salunkhe (1981). A 1 g starch sample was combined with 10 mL of water and stirred for 30 s. Next, the mixture was centrifuged at 4000 rpm for 30 min after being left at ambient temperature for 30 min. The sample's water absorption was ascertained utilizing equation 6.

$$\text{Water absorption (g/g)} = \frac{\text{Weight of water (g)} - \text{Weight of supernatant (g)}}{\text{weight of the sample (g)}} \quad (6)$$

2.3.10 Starch gelatinization profile analysis

The starch's gelatinization profile was assessed utilizing a Rapid Visco Analyzer (RVA). A 3.5 g starch sample was combined with 25 mL of water in an RVA container. The sample mixture was then stirred at 160 rpm while being heated to 50°C and held for 1 min. The temperature was elevated to 95°C at a rate of 12.2°C/min and sustained at 95°C for 2.5 min. The temperature was ultimately decreased to 50°C at a rate of 1.2°C/min and sustained at that temperature for 2.1 min.

2.3.11 Experimental design and statistical analysis

The study used a completely randomized design with two treatment factors: temperature variation (105, 120, and 135°C) and autoclaving period (20, 40, and 60 min). Each treatment was done with three replicates. The RS data were analyzed using two-way ANOVA ($P=0.05$) with the Duncan multiple range test (DMRT) as a post-hoc analysis. Other data were analyzed using the T-test. Microsoft Excel and IBM® SPSS® Statistics 22.0 were used for data collection and statistical analysis.

3. Results and Discussion

3.1 RS content of modified arrowroot starch

RS comprises a category of starch and its derivatives that remain indigestible within the gastrointestinal tract (Nigudkar, 2014). Arrowroot starch possesses natural RS and high amylose content, providing as a source of RS (Purba, 2007; Sugiyono et al., 2009; Faridah et al., 2014; Maulani & Hidayat, 2016). This study examined the autoclaving-cooling method for the physical modification of arrowroot starch to enhance its RS content. As

listed in Table 1, the temperature and duration of autoclaving significantly influenced the development of RS during the autoclaving-cooling process. Both the temperature and duration of autoclaving affected the degree of starch gelatinization. Elevated autoclaving temperatures can disrupt a greater number of amylose intermolecular interactions (hydrogen bonds), thereby facilitating the dissolution and increased release of amylose from the granules (Xie et al., 2006; Li et al., 2015; Guo et al., 2018; Xing et al., 2018; Faridah et al., 2022). A greater release of amylose from the granules during gelatinization results in increased re-association of amylose to create the RS double helix structure (Zakiyah et al., 2010; Rosida et al., 2015). Prolonged autoclaving time can contribute to more complete starch gelatinization, resulting in the release of more amylose molecules, which in turn facilitates the reassociation of amylose molecules during retrogradation (Song et al., 2012; Lilia-Baby et al., 2016). The length of the heating process determines the damage to the crystalline structure of starch and the degradation of starch macromolecules (Guha & Ali, 2002; Huang et al., 2022).

Based on the data presented in Table 1, all modification treatments (temperature and autoclaving time) in this investigation resulted in an increase in the RS content of modified arrowroot starch. The RS content of modified starch ranged from 1.48% to 5.02% d.b., whereas the RS value of native starch was 1.23% d.b. The highest RS content was obtained at 105°C for 40 min, while the lowest RS content was observed at 135°C for 60 min.

Starch undergoes gelatinization during the autoclave procedure, wherein the amylose chains are released from the starch granules in the form of random coils. In contrast, the retrogradation process in starch results in the recrystallization of the amylose chains into a tight double helix configuration that is stabilized by hydrogen bonds. Therefore, the concentration of RS progressively increased as the autoclaving duration increased. Nonetheless, elevating the temperature and prolonging the gelatinization duration would lead to a reduction of RS. The study findings indicated that the lowest RS in arrowroot starch was generated under autoclaving conditions at the maximum temperature of 135°C and the longest duration of 60 min, yielding an RS value of 1.48% d.b.

Table 1. RS content of modified arrowroot starch

RS Content of Modified Arrowroot Starch (% d.b.)			
Autoclaving Temperature (°C)	**Autoclaving Time**		
	20 min	**40 min**	**60 min**
105°C	4.82^{Cb}±0.03	5.02^{Cb}±0.12	4.25^{Ba}±0.06
120°C	2.56^{Bc}±0.08	2.13^{Bb}±0.22	1.59^{Aa}±0.04
135°C	1.52^{Aa}±0.07	1.51^{Aa}±0.15	1.48^{Aa}±0.10

Note: Different superscript capital letters indicate different values within the same column. Different superscript lower case letters show different values within the same row.

This finding differs from those of prior investigations. Arrowroot starch was modified with 5 cycles of autoclaving cooling (15 min; 121°C) to obtain 12% RS. However, the study found that the longer the autoclaving period, the lower the level of RS produced (data not shown), with an autoclaving duration of 30 min at the same temperature (Sugiyono et al., 2009). The reduction in RS concentration with elevated autoclaving temperature and duration is associated with the hydrolysis process that may occur during autoclaving. An excessively prolonged autoclaving process would enhance hydrolysis, resulting in the production of amylose with a relatively reduced degree of polymerization (DP). The degree of starch polymerization decreased as the autoclaving time increased, and the number of hydrolysis phenomena increased (Zheng et al., 2020). Elevated autoclaving temperature and duration enhanced the degradation of amylose chains, leading to an abundance of short-chain amylose (low DP) (Guha & Ali, 2002). The degree of polymerization of amylose varies according to the type of substance. The average DP of arrowroot starch amylose is 2,840, which is lower than that of cassava starch amylose (4,000) and potato amylose (4,450). The reduced DP of arrowroot starch amylose facilitates the production of excessive amylose with a suboptimal DP following the high-temperature autoclaving procedure, rendering it ineffective in building the RS double helix structure (Stevenson, 2003). Research by Leong et al. (2007) indicated that high-temperature thermal processes, such as autoclaving, resulted in the degradation of sago starch, yielding a population of short-chain starches. Similarly, Mutungi et al. (2009) demonstrated that autoclaving treatment of modified cassava starch resulted in the depolymerization of cassava starch, which led to the formation of lower molecular weight molecules (short-chain starch).

3.2 Characteristics of the highest RS of modified arrowroot starch

The modified arrowroot starch, which exhibited the highest RS content following autoclaving at 105°C for 40 min, was subsequently characterized for its physical, chemical, and functional properties.

3.2.1 Physical properties

The physical modification process of starch not only altered the chemical composition of starch but also affected its physical properties. Notable changes were observed in the physical properties such as color and bulk density. The physical properties of both native and modified arrowroot starch are shown in Table 2.

Table 2. Physical properties of native and modified arrowroot starch

Physical Properties	Native Starch	Modified Starch
*L Value	88.00^{b}±1.00	83.33^{a}±1.10
*a Value	1.00^{b}±0.18	0.02^{a}±0.01
*b Value	6.66^{a}±0.79	13.00^{b}±1.00
ΔE Value	0.05^{a}±0.04	7.96^{b}±0.22
Bulk density (g/mL)	0.69^{ns}±0.06	0.68^{ns}±0.02

Note: Different superscript letters indicate significantly different values within the same row. ns superscript letters indicate no significant difference.

1) Color

The autoclaving-cooling modification process of arrowroot starch influenced its color. The total color difference (ΔE*) between the modified arrowroot starch and the standard native starch was 7.94. The ΔE* value obtained in this study was lower than that reported in a similar study by Wardana and Yulia (2018), who investigated the autoclaving-cooling modification of purple sweet potato starch. In their study, the modified purple sweet potato starch exhibited a ΔE* value of 16.15. A greater ΔE* value indicates a more pronounced color difference between the sample and the standard, suggesting a more significant color change. Therefore, the results of this study show that the autoclaving-cooling treatment of arrowroot starch led to a less noticeable color change.

In comparison to native starch, modified starch exhibited a diminished L* value (brightness) and an elevated +b* value (indicating a greater yellow color inclination). The prolonged drying duration of the modified starch (about 36-40 h) was likely responsible for these alterations. Despite being dried at the relatively moderate temperature of 50°C, an extended drying period may have influenced the sample's color. The reduction in brightness and the increased yellowish hue of the modified starch could be attributed to the non-enzymatic browning, specifically the Maillard reaction (Pizzoferrato et al., 1998). This reaction happens between reducing sugars and amine groups (derived from peptide), resulting in the formation of melanoidin, which exhibits a brownish (dark) hue. The Maillard reaction occurs when polymeric carbohydrates are thermally reduced into monomers or at least dimers. The autoclaving modification process, which involves elevated temperatures, induces the depolymerization of starch into smaller molecular weight molecules, thereby facilitating the creation of sugar monomers or dimers that contribute to the Maillard reaction (Mutungi et al., 2009). Moreover, arrowroot starch comprises a small quantity of protein, depending upon the starch's purity level. Although the protein level is minimal, the protein within the starch cannot be completely eliminated, thus allowing the Maillard process to occur (Mariati, 2001).

2) Bulk density

Bulk density refers to the ratio of the mass of a material (g) to the volume it occupies (mL). It is a crucial physical attribute of the material for assessing the volume of processing equipment, transportation vehicles, and storage facilities (Atmaka & Amanto, 2010). The bulk density serves as an indicator of a material's capacity to occupy a given volume. A lower bulk density of the substance indicates a reduced quantity of material inside the same volume (Efendi et al., 2015).

The bulk density of native arrowroot starch obtained in this investigation was 0.6822 g/mL, which was in agreement with the findings of Mariati (2001), who reported a value of 0.6523 g/mL, and Zakiyah et al. (2010), who reported 0.6420 g/mL. According to Table 2, no significant differences were observed between the bulk density of both native and modified arrowroot starch. The bulk density of modified arrowroot starch was 0.68 g/mL, when the native starch was 0.69 g/mL. Theoretically, heating-cooling cycle treatments can lead to a reduction in bulk density. The autoclaving-cooling process can damage the structure of starch granules and form an irregular structure with a porous network (Babu & Parimalavalli, 2013; Rosida et al., 2015; Jagannadham et al., 2016; Gorecki et al., 2018). Particles with increased porosity creates air-filled cavities, hence reducing bulk density (Atmaka & Amanto, 2010). Materials of same mass may exhibit a lower bulk density due to their necessity for more spatial volume compared to materials

having a higher bulk density. Materials possessing a high bulk density exhibit reduced efficiency in storage, packaging, and transportation (Patria et al., 2013).

3.2.2 Chemical properties

The chemical properties evaluated in this study included moisture content, starch, amylose, amylopectin, ash, and crude fiber. Table 3 displays the chemical properties of both native and modified starch.

Table 3. Chemical properties of native arrowroot starch and modified arrowroot starch

Chemical Properties	Native Starch	Modified Starch
Rs content (%d.b.)	1.24^{a}±0.09	5.02^{b}±0.22
Moisture content (%w.b.)	7.37^{b}±0.02	10.49^{b}±0.15
Starch content (%d.b.)	87.29^{b}±0.20	86.75^{a}±0.25
Amylose content (%d.b.)	25.43^{a}±0.20	27.93^{b}±0.40
Amylopectin content (%d.b.)	61.79^{b}±0.23	58.82^{a}±0.24
Ash content (%d.b.)	0.33^{ns}±0.01	0.36^{ns}±0.00
Crude fiber content (%d.b.)	0.22^{a}±0.00	0.26^{b}±0.00

Note: Different superscript letters within the same row indicate significantly different values. ns superscript letter indicates no significant difference.

1) Moisture content

The moisture content of the modified starch (10.49%) was higher than that of native starch (7.37%), as indicated by the results in Table 3. This variation in moisture content can be attributed to the high initial moisture content during the preparation of modified starch, which involved dissolving the starch in distilled water to form a suspension with a 20% (b/v) concentration. Additionally, the Codex Alimentarius Commission has established a maximum moisture limit of 14% for both native and modified starch used in food applications (NguyenStarch, 2025). This standard is also equivalent to the maximum moisture content specified for starches by the Indonesian National Standards (BSN, 1992).

2) Starch content

The starch content is a critical quality parameter in starch-based products (Mariati, 2001; Prabowo & Ibdal, 2023). The purity of extracted starch depends on how much starch is present. The purity of starch is significantly affected by the starch extraction method. A study by Mariati (2001) revealed that the starch concentration of arrowroot starch varied between 92.24 and 98.78% (d.b). In comparison, the native arrow starch obtained in this study exhibited a lower starch content of 87.29% (d.b). It is due to the suboptimal extraction procedures used in this study. Suboptimal starch extraction can result in several contaminants in the starch and significant starch loss (Fetriyuna et al., 2017).

Table 3 shows that the starch content of modified starch (86.75%) was lower than native starch (87.29%). The reduction in starch content in modified starch seemed to come from the autoclaving process, which stimulated starch degradation, leading to the formation

of lower molecular weight compounds (Mutungi et al., 2009). These small molecules may have dissolved and been lost during the preliminary assessment of starch content prior to acid hydrolysis, resulting in a lower final starch content. Setiarto et al. (2018) also demonstrated a reduction in starch content following autoclaving-cooling modification in taro flour and sorghum flour. The breakdown of the amylopectin component of starch after autoclaving may cause the overall starch content to decrease but the amylose content to rise. Amylopectin, a branching polymer found in starch, is more sensitive to breakdown when exposed to high temperatures and pressures. The increased amylose content could be attributed to the partial breakdown of amylopectin into smaller, more linear chains, but the starch content falls due to the loss of some starch granules (Kim et al., 2006; Li et al., 2020a; Nithya et al., 2024; Sangwongchai et al., 2024).

3) Amylose and amylopectin content

Amylose and amylopectin serve as the primary constituents of starch. Amylose is a linear polysaccharide composed of glucose units linked by α-1,4 glycosidic bonds, whereas amylopectin is a polysaccharide that features both a straight chain, linked by α-1,4 glycosidic bonds, and a branched chain, connected through α-1,6 glycosidic bonds. The functional characteristics of starch are influenced by the composition of amylose and amylopectin (Sajilata et al., 2006). Amylose exhibits a linear structure, which results in a higher propensity for retrogradation, leading to the formation of a double helix structure (RS III), in contrast to the more branched structure of amylopectin (Birt et al., 2013). As shown in Table 3, the amylose concentration of native arrowroot starch in this study was 25.43%. This value was comparable to the findings of Stevenson (2003), who reported an amylose content of 25.6%, and Faridah et al. (2014), who reported 24.64% The amylose content of modified starch (27.93%) in this research was higher than the native starch (25.43%). This was due to the repeated heating and retrogradation processes that occur during autoclaving-cooling. The autoclaving-cooling approach improves amylose concentration in starch by encouraging retrogradation, a process in which starch molecules, particularly amylose, re-associate into a more organized, crystalline structure when cooled after heating. This process effectively converts some of the digestible starch into resistant starch (RS), which is less easily digested in the small intestine (Faridah et al., 2022; Surawan et al., 2024; Wira et al., 2025).

The amylopectin content was determined by subtracting the amylose content from the total starch content. The amylopectin concentration of the native arrowroot starch was 61.79%. According to Table 3, the amylose content of modified arrowroot starch (27.93%) was higher that of native arrowroot starch (25.43%), whereas the amylopectin content exhibited the opposite trend. The amylopectin concentration in modified arrowroot starch (58.82%) was lower than that in native arrowroot starch (61.79%). The rise in amylose content and the decline in amylopectin content in modified starch were attributed to the autoclaving process, which destroyed some of the branching of amylopectin, converting it into linear amylose of reduced molecular weight and shorter chain length (Triwitono et al., 2017; Setiarto et al., 2018). Similar findings were reported in the alteration of sweet potato and banana starch. Autoclaving-cooling modification elevated the amylose content of sweet potato starch from 18.17% to 24.98% (Babu & Parimalavalli, 2013). A similar trend was observed in banana starch, where amylose content increased from 37% to 44.8% following autoclaving-cooling treatment (Jiménez-Domínguez et al., 2015).

4) Ash content

The ash content of native arrowroot starch (0.33%) was not significantly different from that of modified starch (0.36%). These results show that the autoclaving-cooling treatment did not affect the ash content of the modified starch (Aparicio-Saguilán et al., 2005; Babu & Parimalavalli, 2013). This might arise from the autoclaving temperature employed in the modification procedure being insufficient to destroy the minerals in the starch (Aparicio-Saguilán et al., 2005: Anggraini et al., 2023).

5) Crude fiber content

Starch modification through autoclaving-cooling method affected the crude fiber content produced. The crude fiber content of modified arrowroot starch (0.26%) was significantly higher than native starch (0.22%). The crude fiber content in native starch was consistent with the findings of Mariati (2001), who reported the crude fiber content in native arrowroot starch ranged from 0.19-0.50%. Nonetheless, the findings of this research was in contrast to those presented by Adebowale et al. (2005), whose research on the alteration of breadfruit starch through heat moisture treatment, oxidation, and acetylation methods demonstrated a reduction in crude fiber content. Autoclaving and cooling starch can boost its crude fiber content, especially by encouraging the production of resistant starch (RS). This process alters the structure of the starch, resulting in increased crystallinity and retrogradation, which are important characteristics of RS and can contribute to a greater crude fiber content (Pratiwi et al., 2015; Strozyk et al., 2022; Isra et al., 2023).

3.2.3 Functional properties

The functional properties of starch observed in this study were swelling power, solubility, water absorption, and starch gelatinization profile. Table 4 shows the result of swelling power, solubility, as well as water absorption.

Table 4. Swelling power, solubility, and water absorption of native arrowroot starch and modified arrowroot starch

Functional Properties	Native Starch	Modified Starch
Swelling power (g/g)	8.24^{b}±0.28	2.24^{a}±0.18
Solubility (%)	4.09^{b}±0.24	1.82^{a}±0.11
Water absorption (g/g)	1.62^{b}±0.08	0.83^{a}±0.09

Note: Different superscript letters within the same row indicate significantly different values.

1) Swelling power and solubility

Swelling power indicates the degree of swelling of starch granules during heating in the presence of water. It also an indicator of hydration capacity of starch (Shimelis et al., 2006; Zhu, 2014). While solubility represents the percentage of starch dissolved in the supernatant (Kaur et al., 2011). Both swelling power and starch solubility indicate the level of interaction in the amorphous and crystalline structures of starch granules (Alexander et al., 2024). In this study, the swelling power of native arrowroot starch (8.24 g/g) was found

to be significantly higher than modified starch (2.24 g/g). A similar trend was observed in the starch solubility results, with native arrowroot starch exhibiting a solubility of 4.09%, compared to 1.82% for modified starch. The results indicated that autoclaving-cooling treatment caused a decrease in swelling power and solubility. This reduction can be attributed to the re-association of amylose after the modification process. During autoclaving, starch granules develop a novel double helix structure, characterized by strengthened intermolecular connections, resulting in an increased level of starch crystallinity (Alcázar-Alay & Meireles, 2015). The improvement in starch crystallinity affects the disruption of hydrogen bonds among starch molecules, hence hindering water's interaction with starch. Consequently, this results in decreased swelling power and solubility values in modified starch (Kasote et al., 2018; Syafutri et al., 2018).

Swelling power indicates water-holding ability of starch during heating and is an important feature of the interaction between amylose and amylopectin. Water holding capacity of starch is related to the presence of hydrogen bonds between its chains. The quantity of hydrogen bonds and covalent interactions between starch chains is inversely correlated to the starch's ability to bind water. As a result, the more hydrogen bonds and covalent interactions between starch chains, the less starch can bind water, resulting in a lower capacity to retain water (Li et al., 2020b; Zhang et al., 2021). Autoclaving disrupts the hydrogen interaction between starch chains, allowing water to enter the starch chains and compete for hydrogen bonds, boosting water-holding capacity. A similar phenomenon was reported with *Canna edulis* starch (Zhang et al., 2021). Autoclaving caused a significant decrease in solubility and swelling power, likely due to amylose release and alterations in amylose-amylose and amylose-amylopectin interactions. Similar findings were reported on ginger starch following autoclaving-cooling treatment (Li et al., 2021; Zhang et al., 2021). Reduced swelling power and solubility were also found on taro starch following autoclaving-cooling modification (Wiadnyani et al., 2015). Comparable findings were observed for modified rice starch using autoclaving (Roushdi et al., 2016). Similarly, chemical alteration of oat starch through cross-linking modification with citric acid resulted in reduced swelling power and solubility values (Alexander et al., 2024).

2) Water absorption capacity

Water absorption capacity describes the ability of a material to associate with water under limited water conditions (Ali et al., 2016). This parameter is important as the nature of the starch system is influenced by the amount of water added to the starch. Water absorption capacity represents the amount of water absorbed per unit of mass of starch samples (Mariati, 2001). The water absorption of native arrowroot starch (1.62 g/g) was found to be significantly higher than modified arrowroot starch (0.83 g/g). The autoclaving-cooling treatment caused a decrease in water absorption in modified starch. These findings were consistent with previous studies. For instance, autoclaving treatment of edible tapioca pearls showed a decrease in water absorption (Kasote et al., 2018). The ability of starch to absorb water is influenced by its internal structure, particularly the presence of hydroxyl groups in forming hydrogen bonds between starch molecules. Stronger hydrogen bonds between starch molecules can result in lower water absorption (Ali et al., 2016; Marta & Tensiska, 2017). The crystallinity level of modified starch is higher than native starch because it has a double helix internal structure with stronger intermolecular hydrogen bonds. This structural change is thought to be responsible for the low water absorption in modified starch compared to native starch.

3) Starch gelatinization profile

Starch gelatinization profile in this study was analyzed using Rapid Visco Analyzer (RVA). The gelatinization profile graph of native arrowroot starch and modified arrowroot starch is shown in Figure 1. Based on Figure 1, the modified arrowroot starch exhibited a lower peak viscosity compared to native starch. This showed that the autoclaving-cooling process caused a decrease in viscosity in the modified arrowroot starch. Similar findings were reported in previous studies, where the autoclaving-cooling process caused a decrease in viscosity of modified chickpea starch (Jagannadham et al., 2016). The gelatinization profile of both native and modified arrowroot starch is summarized in Table 5.

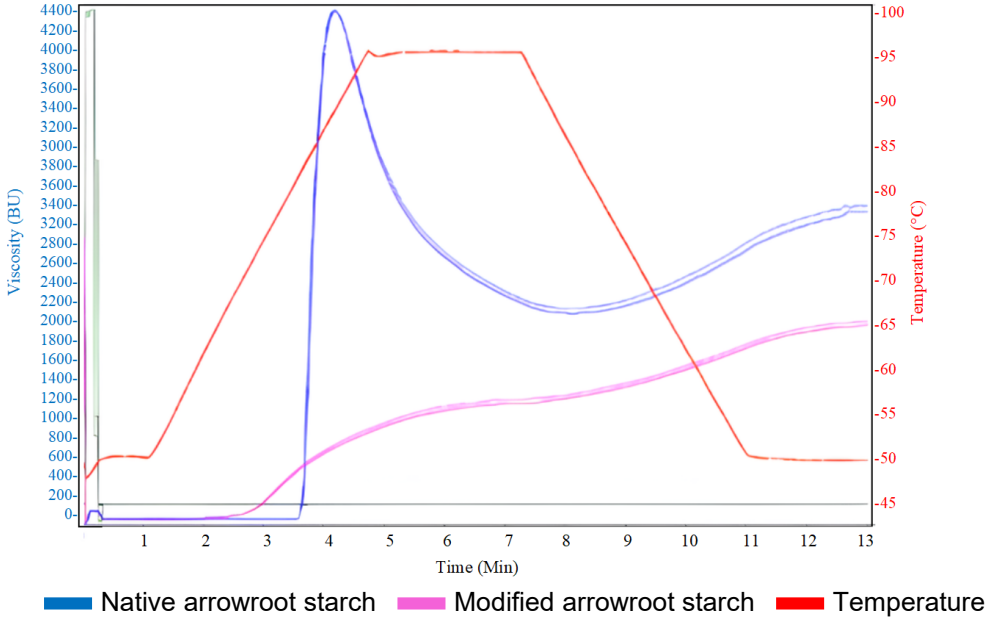

Figure 1. Gelatinization profile graph of native and modified arrowroot starch

Table 5. Gelatinization profile of native arrowroot starch and modified arrowroot starch

Gelatinization Profile	Native Starch	Modified Starch
Peak viscosity (cP)	4361.5^{b}±7.77	1016^{a}±4.24
Trough viscosity (cP)	1793.5^{b}±6.36	964^{a}±2.83
Breakdown viscosity (cP)	2568^{b}±2.83	52^{a}±2.83
Final viscosity (cP)	2666.5^{b}±2.83	1684^{a}±2.83
Setback viscosity (cP)	873^{b}±2.83	720^{a}±2.83
Peak time (min)	4.2^{ns}±0.14	7^{ns}±1.41
Pasting temperature (°C)	80.73^{ns}±2.83	71.7^{ns}±2.54

Note: Different superscript letters within the same row indicate significantly different values. ns superscript letter indicates no significant difference.

Peak viscosity occurred when the highest viscosity was reached during heating at 95°C. It represents the maximum viscosity value attained prior to the rupture of starch granules, which occurs due to their inability to retain the water infiltrating the granules. The maximum viscosity value correlates with the swelling power of the starch (Shafie et al., 2016). A low peak viscosity indicates that the starch granules did not swell to their full potential after heating (Rosida et al., 2017). As shown in Table 5, the peak viscosity of modified starch was lower than native starch. This reduction in peak viscosity can be attributed to the internal structure of the modified starch, which resists water binding, resulting in a lower swelling power compared to native starch (Kasote et al., 2018; Syafutri et al., 2018). A low peak viscosity may imply a reduced water absorption capacity and swelling power of the starch granules. This can be useful in goods that require a less viscous or thick texture, such as some types of sauces or gravies, or when excessive thickening caused by starch swelling is undesired (Braşoveanu & Nemţanu, 2020).

Trough viscosity was obtained when the lowest viscosity is achieved during heating at a temperature of 95°C. It measures the lowest value achieved after the rupture of starch granules (Shafie et al., 2016). The difference between the peak viscosity value and the trough viscosity is the breakdown of viscosity. This parameter reflects the stability of the starch paste during the heating process. A lower breakdown viscosity value suggests greater stability of the starch paste to heating and stirring (Pomeranz, 1991; Shafie et al., 2016). Conversely, a higher breakdown viscosity indicates that the swollen starch granules are more fragile and susceptible to destruction by heating and stirring (Pomeranz, 1991; Sangwongchai et al., 2024).

Breakdown viscosity is the difference between peak and trough viscosity (the lowest viscosity following the peak). It shows the starch paste's resilience to shear stress and heat. As shown in Table 5, modified starch exhibited a lower breakdown viscosity than native starch. A lower breakdown viscosity indicates a more stable starch paste that is less likely to break down or disintegrate when subjected to shear pressures during processing. This is useful in situations where the paste must keep its structure while mixing, pumping, or other mechanical processes (Faridah et al., 2022). The RS type III formed during the autoclaving-cooling modification process is known to be highly heat stable with a melting temperature of around 150°C (Kim & Kwak, 2009). This increased heat stability may explain the lower breakdown viscosity observed in the modified starch.

Final viscosity refers to the viscosity obtained when cooling at 50°C. It measures the ability of starch to form a viscous paste after the heating and cooling process (Shafie et al., 2016; Rosida et al., 2017). In this research, the final viscosity of modified starch was found to be lower than native starch. The viscosity value of starch plays a crucial role in determining its suitability for various applications in the food industry. Modified starch with low viscosity is unable to form a good viscosity paste, making it less suitable for use as the main ingredient (100% composition) in products such as noodles and bread. Instead, it must be combined as a composite with wheat flour and other flour. The viscosity of modified arrowroot starch (1016 cP of peak viscosity and 1684 cP of final viscosity) was comparable to the viscosity of whole wheat flour which had a peak viscosity of 1329 cP and a final viscosity of 1647.5 cP (Kundu et al., 2016). This suggests that modified arrowroot starch can be applied in products that typically use whole wheat flour as a composite, such as noodles (80% composition) (Cao et al., 2017) and bread (20% composition) (Ngozi, 2014).

A decreased final viscosity in modified starch, on the other hand, has various benefits, the most important of which are enhanced processing and textural properties in food items. It can result in improved flow qualities, simpler inclusion into formulations, and a smoother, less viscous finished product. Lower viscosity means the modified starch flows more smoothly, making it easier to handle and incorporate into various food processing applications like pumping, mixing, and filling. Starch with a reduced viscosity can help save energy during processing since it requires less force to move. Modified starches with decreased viscosity disperse more easily in liquids, resulting in more homogeneous mixtures and preventing clumping or sedimentation. In many applications, a lower final viscosity yields a smoother, less pasty texture in the completed product, which may be more appealing to customers. It can also assist in lessening the stickiness of certain food products, making them more appetizing and convenient to handle. It can avoid a "gummy" or excessively thick mouthfeel, especially in items like sauces, soups, or fillings (Iida et al., 2008; Bravo-Núñez et al., 2019).

Setback viscosity is obtained from the subtraction between the final viscosity and the trough viscosity. It shows the tendency of starch to undergo retrogradation after gelatinization and cooling at 50°C. During cooling process, starch molecules, especially amylose, tend to re-associate and undergo retrogradation (Ragaee & Abdel-Aal, 2006). The lower the setback viscosity value, the lower the tendency of starch retrogradation can be (Shafie et al., 2016). Lower setback viscosity implies a lower tendency for retrogradation and a more stable cooked paste over time. This can help prevent undesirable changes in texture, such as staling or syneresis (separation of liquid from the gel), during storage. Table 5 shows that the setback viscosity of modified starch was significantly lower than native starch. Modified starch has a double helix structure with strong intermolecular bonds, which make it difficult to re-associate amylose during retrogradation and produce a low level of retrogradation. Research by Sun et al. (2014) demonstrated that interactions between sugar molecules, amylose, and amylopectin in starch cause a decrease in the level of amylose leaching during gelatinization and make it more difficult for amylose to re-associate, thereby resulting in a lower level of retrogradation. Starch with a high retrogradation tendency is considered undesirable for application in food products because it can adversely affect the texture of starch-based products (Rosida et al., 2017).

Peak time is the time when starch experiences the highest peak viscosity. It represents the time required for starch granules to break because of their inability to hold the water entering the granules. The peak time of modified starch was observed to be higher than that of native starch (Table 5). This could be caused by the lower water absorption capacity of modified starch, which takes a longer time to reach peak viscosity compared to native starch. The decrease in water penetration in starch molecules during the heating process may delay the peak time and result in a higher peak time (Zhang et al., 2017).

Pasting temperature refers to the temperature when viscosity begins to increase during the heating process. Starch with high pasting temperature indicates that the starch is more resistant to swell (Kumar & Khatkar, 2017). The results presented in Table 5 show that the pasting temperature of modified starch was lower than that of native starch. These observations were in agreement with Sangwongchai (2024). Waxy and non-waxy rice starches modified through autoclaving-cooling treatment showed a decrease in pasting properties and a decrease in final viscosity. This phenomenon likely occurred because the autoclaving-cooling cycle treatment enhanced the development of the amylopectin short-chain area by degrading the amylopectin long-chain region during autoclaving. The augmented amylopectin short-chain area, characterized by unique and irregular short branch chains, may obstruct the molecular connections among the starch chains dispersed

in gelatinized starch after cooling (Hendry et al., 2022; Sangwongchai et al., 2024; Yonata et al., 2025).

Overall, the modifications made in this study reduced all pasting properties. The reduction in the pasting properties profile was directly proportional to the decrease in swelling power. In theory, adjustments to autoclaving and cooling can enhance trough viscosity and setback viscosity. Autoclaving-cooling procedures enhance starch stability, yielding increased trough viscosity during the pasting process. The cooling phase of the cycle facilitates retrogradation, resulting in an increased setback viscosity (Rosida et al., 2017; Surawan et al., 2024).

Modified starch through hydrothermal processing also exhibited higher pasting temperature due to the increased energy required to disrupt the starch granule. The higher energy requirement was attributed to the stronger intermolecular bonds present in modified starch (Chen et al., 1998; Marta & Tensiska, 2017). Additionally, other studies on modified starch using autoclaving-cooling procedure were unable to detect a pasting temperature (Polesi & Sarmento, 2011; Reddy et al., 2013; Rosida et al., 2017). The undetectable pasting temperature may have been the result of the viscosity increasing at a constant rate for a long time (Bao, 2008). This phenomenon could be attributed to the disruption of starch granules during the autoclaving process (Reddy et al., 2013).

The starch modification process, involving autoclaving-cooling, can change the starch gelatinization profile. Based on this profile, native arrowroot starch is classified under the type A gelatinization profile, which is characterized by a high swelling ability (Faridah et al., 2013). A type A gelatinization profile is indicated by a high peak viscosity, followed by a sharp decrease in viscosity during heating (Schoch & Maywald, 1968). Meanwhile, modified arrowroot starch falls under the type C gelatinization profile, which has limited swelling ability. Starch with a type C gelatinization profile starch tends not to have a peak viscosity, but instead show a high viscosity and either constant or increasing viscosity during heating (Schoch & Maywald, 1968).

4. Conclusions

Based on the results of this study, the modified arrowroot starch with the highest RS content was obtained at an autoclaving temperature of 105°C and a duration of 40 min. The chemical characteristics of the modified arrowroot starch met the standards set by SNI (Indonesian National Standard): moisture content (10.49%), starch (86.75%), amylose (27.930%), amylopectin (58.82%), ash (0.36%, crude fiber (0.26%). The results of the physical and functional characteristic analysis were color brightness (L*) (83.33), density (0.69 g/ml), swelling power (2.24 g/g), solubility (1.82%), water absorption (0.83 g/g), and better paste stability. The amylograph curves of modified starch indicated that the autoclaving-cooling modification process led to a more stable pasting profile. These findings demonstrate that the autoclaving-cooling process significantly enhanced the RS content, even though it caused a reduction in the functional properties of the starch.

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6. Authors' Contributions

All authors contributed equally to this paper. All writers reviewed and endorsed the final manuscript. Judella Kusuma Halim: Writing (review and editing), writing (original draft), formal analysis. Salsa Gena Aldama: Investigation, composition (review and editing). Aura Fitri Noviandari: Writing (review and editing), writing (original composition), formal analysis. Tengku Farizan Izzi Binti Che Ku Jusoh: Authorship (review and editing), authorship (original draft), research, formal analysis. Aprilia Fitriani: Investigation, writing (review and editing), supervision, conceptualization, financing acquisition. Nurul Putrie Utami: Authorship (review and editing), authorship (original draft), research. Nurul Hidayah: Authorship (review and editing), authorship (original draft), data analysis.

7. Conflicts of Interest

The authors declare that they have no conflicts of interest to declare.

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