

PHYSICOCHEMICAL AND FUNCTIONAL PROPERTIES OF RS3 OBTAINED FROM CILACAP BREADFRUIT (*Artocarpus altilis* (Parkinson) Fosberg) STARCH

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Abstract

Cilacap breadfruit is a variety native to Indonesia, characterized by superior properties, including fast growth, large fruit size, high carbohydrate, and starch content. This plant has a relatively high content of amylose and resistant starch (RS), allowing its conversion into RS for health benefits. Therefore, this study aimed to investigate the effect of autoclaving temperature (100, 121, and 140°C), cooling at 4°C, and storing time (24 and 48 hours) on the formation of RS from Cilacap breadfruit, and determine its physicochemical (amylose, amylopectin, resistant starch, pasting properties, and thermal properties), as well as functional properties. The results showed that autoclaving-cooling increased the amylose and RS content, water solubility, onset, and peak of thermal temperature. The pasting properties of all parameters, gelatinization enthalpy, swelling power, and amylopectin content were decreased. Amylose content also significantly increased ($p \leq 0.05$) from 31.01 ± 0.24 to 37.91 ± 0.43 , and RS content ($p \leq 0.05$) from 10.98 ± 0.07 to 19.41 ± 0.05 . Furthermore, native starch and Cilacap breadfruit RS3 were categorized as high in Amylose. The autoclaving cooling process on Cilacap breadfruit starch effectively increased RS levels, making it a viable alternative for developing functional food

Keywords: Cilacap breadfruit, Resistant Starch type 3 (RS3), Autoclaving-cooling, Physicochemical characteristics, Functional characteristics

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Introduction

Carbohydrates are calorie sources found in various botanical plants across the world such as ripe breadfruit, containing approximately 76.7% (Tan *et al.*, 2017) to 84% (Kazeem *et al.*, 2018). Furthermore, their major constituents are starch, which occupies more than 60% of total carbohydrates (Kazeem *et al.*, 2018). In Indonesia, cilacap breadfruit starch is one of the biological potentials developed as food for agricultural commodities with health benefits (Praseptianga *et al.*, 2018; Rahayu *et al.*, 2021). This is due to several beneficial properties of Cilacap breadfruit, such as fast growth, high yield with two harvests, an average production of 200 to 300 fruits, large fruit size, high carbohydrates, starch, and fiber content (Rahayu *et al.*, 2021).

Starch is a complex carbohydrate, consisting of two main macromolecules, namely amylose, and amylopectin, which differ based on the constituent chains difference (Ai *et al.*, 2013a; Tan *et al.*, 2017). Amylose is a polysaccharide starch constituent, majorly composed of linear molecules interconnected by α -1, 4 D-glucopyranose units with a few α -1,6 linkage branch chains. Meanwhile, amylopectin is composed of several branched chain molecules with α -1,6 bonds. The starch in food has many functions mainly serving as an energy source, a stabilizer, and being used for thickening, consistency, and gelling (Ai *et al.*, 2013a; Reddy *et al.*, 2014). The amylose-amylopectin ratio of starch varies according to the botanical source (Ashwar *et al.*, 2016).

Starches are grouped into three types according to their digestibility by enzymes in the small intestine, namely resistant starch (RS), slow-digesting starch (SDS), and Rapid-digesting starch (RDS). RS is characterized by resistance to hydrolysis, remaining undigested after 120 min, passing from the small to the large intestine. In contrast, SDS digests in vitro between 20 to 120 min, while RDS is digested quickly, causing a rapid increase in blood glucose level, within 20 min (Englyst *et al.*, 1992). The demand for resistant starch to reduce the glycemic index and obtain beneficial health has increased due to the rising prevalence of people with obesity, insulin resistance, diabetes, and metabolic syndromes (Ai *et al.*, 2013b). A previous study has identified the significance of RS in modulating the glycemic index, managing body weight, lowering cholesterol, and preventing diabetes (Nasrin and Anal, 2014). The five classifications of RS included RS1 (physically inaccessible starches), RS2 (native or non-gelatinized starch granules), RS3 (retrograded

starches), RS4 (chemically modified starches), and RS5 (amylose-lipid complex). Among these RS, RS3 has high durability under various processing conditions, particularly at high-temperature, while maintaining nutritional characteristics (Nasrin and Anal, 2014). The RS3 has been successfully produced using various starches from local botanical sources such as processing elephant foot yam (Reddy *et al.*, 2014) and cowpea by autoclaving-cooling method (Ratnaningsih *et al.*, 2020), and breadfruit by heat-moisture treatment (HMT) method (Tan *et al.*, 2017), as well as sago (Rashid *et al.*, 2020), and other starchy plants. Although breadfruit starch has been studied to increase RS, the autoclaving-cooling method followed by acid hydrolysis has not been carried out, particularly on breadfruit originating from the Cilacap district, Indonesia. This breadfruit possesses potential as a functional food ingredient with health benefits.

This study aimed to produce retrograded starch (RS3) from native Cilacap breadfruit starch through autoclaving-cooling cycles, followed by acid hydrolysis, and investigate its physicochemical, and functional characteristics.

Materials and Methods

Breadfruit was used as the main material for breadfruit starch production in this study. The ripe fruit was harvested in September 2018 from a resident's garden in Jetis hamlet, Klepu village, Ceper District, Klaten Regency, Central Java, Indonesia. Furthermore, the ripe fruit was characterized by changes in the color of the fruit peel from light green to dark green-brown, accompanied by white sap stains originating from the latex tube in the fruit (Deivanai and Bhore 2010; Famurewa *et al.*, 2016). The starch was extracted by wet extraction and was used for RS3 preparation. The wet extraction was carried out through several processes, including fruit that is peeling, washing, slicing into small pieces, soaking, and draining the fruit. This was followed by direct grounding for the extraction process, without the fruit drying process.

The white bottom sediment starch modified by the extraction process for isolation with sediment separation was used in this study (Rahayu *et al.*, 2019), as the development of a wet milling method (Nwokocha and Williams, 2011). A total of 12 breadfruits, were peeled and washed thoroughly. Each fruit was sliced into 8 parts, soaked in clean water, drained, weighed, and milled wet using a disc grinding machine (FFC-23A, number 6367, China) at a speed of 5,800 rpm. The pulp was weighed,

followed by the addition of distilled water with a ratio of 1:2 (w/v), and squeezed using a muslin cloth. The condensed starch milk was collected in a 6 ml pan, while the waste was obtained in a container. After all the starch slurry was squeezed, distilled water was added to the accumulated wastes at a ratio of 1:2 (w/v), and deposited for 4 h, followed by supernatant decantation. The pulp squeezing and decantation process were repeated thrice. Distilled water was added to the total starch milk obtained at a ratio of 1:1 (v/v), and the pan was tilted and left for 2 h to allow sedimentation. Subsequently, the top clear liquid in the pan was poured off carefully, and the pan was tilted and left for ± 10 -15 min for the second sedimentation. The top slurry was separated from the bottom sediment by slowly shaking the pan ± 4 times. The upper portion was quickly poured over another stainless-steel pan measuring 30x30x30 cm, and dried in a cabinet dryer (serial number 2015, manufactured by the Wangdi Engineering workshop, Yogyakarta, Indonesia) at 60°C for 2 days, with each session lasting 7 hours. This was followed by grounding, and sifting using a 100 mesh-sieve. The top deposit was brownish, while the bottom sediment was taken quickly using a spoon and placed on a stainless-steel pan (30x30x3 cm). This material was subjected to drying in a cabinet dryer at 60°C for two days, each session lasting for 7 hours, followed by grounding, and sifting using a 100 mesh sieve. The bottom starch deposits were white, with an L^* value of 94.11 (Rahayu *et al.*, 2019), stored in sealed and closed containers for further use.

The RS was prepared according to the method described by (Dundar and Gocmen, 2013) as modified by Rahayu *et al.* (2021). In this process, 150 g of breadfruit starch was placed in a glass beaker and dissolved in 300 mL of distilled water, followed by stirring with a glass stirrer until homogeneous. In another position, 300 mL of distilled water in a glass beaker was heated at 70°C, poured into the starch solution, and stirred with a glass stirrer until homogeneous, where the total ratio of starch: distilled water = 1: 4 (w/v). The starch solution was autoclaved at 100°C, 121°C for 30 min modified by Rahayu *et al.* (2021), and 140°C for 30 min, according to (Dundar and Gocmen, 2013) for the gelatinization process. The mixture was cooled at room temperature for 1 h, and stored at 4°C for 24 and 48 hours (Rahayu *et al.*, 2021), while the autoclaving-cooling process was repeated in 3 cycles. After the completion of the cycles, the starch solids were reduced in size, placed on a stainless-steel pan (30x30x3 cm) to dry in a cabinet dryer at 45°C for 24 hours, ground, and sifted using a 100 mesh sieve. This retrograded starch was put in a beaker glass and a solution of 0.1

M HCl (Starch: HCl = 1: 4 w/v) was added for the incubation process at 40°C for 24 hours in a water bath shaker (Julabo SW22, Germany). Subsequently, the starch was neutralized in 1 M NaOH, the pH was measured with a digital pH meter (HI 2210, Japan), and stored at 4°C for 24 hours. Starch solids were reduced in size, dried on a steel pan (30x30x3 cm) in a cabinet dryer, ground, and sifted using a 100 mesh sieve. RS3 at various autoclaving temperatures (100, 121, and 140°C), and storage times (24 and 48 hours) were stored at 4°C in sealed containers for further use.

Physicochemical analysis was carried out on native starch and Cilacap breadfruit RS3, including amylose content (Williams *et al.*, 1970), RS content (Goñi *et al.*, 1996), pasting and thermal properties. Furthermore, amylopectin content was analyzed differently by subtracting amylose from starch content.

Amylose content was determined based on the method of Williams *et al.* (1970), where 1 mL of 95% ethanol, and 9 mL of 1N NaOH, were added to the starch sample (native starch or Cilacap breadfruit RS3 of 0.1 g). The solution was heated in a water bath at 95°C for 10 minutes and cooled for ± 50 minutes. Subsequently, 5 mL of the solution was taken and transferred to an Erlenmeyer glass (100 mL). A total of 1 mL of acetic acid 1 N, 2 mL of 2% iodine, and 92 mL of distilled water were added to the sample solution and incubated at 20-25°C for 20 minutes. The sample was measured using a UV-Vis spectrophotometer (Shimadzu model UV-1800, Kyoto, Japan) at 625 nm. The amylose was determined by making a comparison with the potato amylose (mg/L) standard curve.

The RS content was analyzed by the Goñi *et al.* (1996) method with modification. In this process, 100 mg of the sample was put in a 25 mL screw-closed tube, followed by the addition of KCl-HCl buffer solution (10 mL, pH 1.5), and stirred. Pepsin solution (1 g in 10 mL KCl-HCl, 0.2 mL) was added, and the mixture was stirred again in a water bath shaker at 40°C and allowed to cool at room temperature. Subsequently, 9 mL Trismaleate buffer (0.1 M, pH 6.9), and α -amylase solution (40 mg α -amylase/mL trismaleate buffer, 1 mL) were added. The mixture produced was incubated in a water bath shaker at 37°C for 16 h, centrifuged at 3000 g for 15 minutes, and the supernatant formed was discarded. A total of 10 mL of distilled water was added to the sediment centrifuged, and the supernatant formed was discarded. Approximately 3 mL of distilled water was added to the residue, mixed with 3 mL KOH 4 M, and stirred using a magnetic stirrer for 30 minutes until homogeneous. A 5.5 mL HCl 2 M and 3 mL sodium acetate buffer (0.4 M, pH 4.75) were added for

neutralization. This was followed by mixing with 80 μ L amyloglucosidase at 60°C, pH 4.8, for 45 minutes until homogeneous. The mixture was incubated in a water bath at 60°C for 45 minutes and centrifuged at 3000 g for 15 minutes. The initial supernatant formed (S1) was collected in a measuring flask of 50 ml, while 10 mL of distilled water was added to the residue, shaken out, and centrifuged. The second supernatant formed (S2) was combined with the initial one (S1). Distilled water was added into a mixture of S1 and S2 until the total volume became 50 mL, resulting in the final mixture referred to as the supernatant (SN). Approximately 20 μ L of glucose-oxidase and peroxidase, abbreviated as GOPOD reagent was added to the SN, stirred using a magnetic stirrer for 15 minutes, and incubated in a water bath at 37°C for 10 minutes. The sample and standard absorbances were read against a reagent blank and measured with a spectrophotometer at 510 nm. The sample glucose concentration was calculated using a standard curve and the resistance starch was estimated as total glucose (mg) $\times$ 0.9, with 0.9 representing a conversion factor for free D-glucose.

The analysis of pasting and thermal properties was carried out on native starch (NS) and Cilacap breadfruit RS3. The measurements of pasting properties were conducted using a Rapid Visco Analyzer (RVA 4500, Perten Instrument, Sweden) (Ratnaningsih *et al.*, 2020). In this process, 3.5 g starch was weighed into the sample container and added with 25 g distilled water. The starch slurry was conditioned at a temperature of 50°C for 1 minute, heated to 95°C at a rate of 6°C/minute, and allowed to stand for 2 minutes. The initial speed for the first 10 seconds was 960 rpm and the remainder was at 160 rpm. Subsequently, the viscoamylographs parameters including peak, trough, breakdown, final, and setback viscosity were obtained.

The thermal properties of the samples were tested using a Differential Scanning Calorimeter (DSC, TA-60WS, Shimadzu, Japan) (Ratnaningsih *et al.*, 2020). Approximately 3.0 mg of the sample was weighed, and put in a standard hermetic aluminum pan, followed by the addition of 10 μ L of distilled water (ratio of starch: distilled water = 1: 3). Before heating in the DSC, the mixture was tightly closed and allowed to stand for 90 min. As a reference, another hermetic pan containing alumina powder (6.0 mg) was used, which was placed next to the sample pan in the DSC chamber. The sample and reference were heated at temperatures from 30°C to 250°C at a speed of 5°C/min. The DSC curve showed the thermal parameters, which included onset temperature (T_o), peak temperature (T_p), end temperature (T_c), and enthalpy of

gelatinization (ΔH), calculated using the TA-60WS software.

Functional characteristics analysis was conducted on native starch (NS) and Cilacap breadfruit RS3, including water solubility (WS), water holding capacity (WHC), and swelling power (SP). Water solubility (WS) of the samples was determined following the procedures from a previous study (Singh and Singh, 2003) with modification by (Dundar and Gocmen, 2013). The NS and Cilacap breadfruit-resistant RS3 were used at 100°C/24 h, 100°C/48 h, 121°C/24 h, 121°C/48 h, 140°C/24 h, and 140°C/48 h. A total of 0.5 g dry sample was put in a test tube, and 5 mL distilled water was added, followed by vortexing for 15 seconds every 5 minutes. After 40 minutes, the mixture was centrifuged at 2100 g for 10 minutes. The supernatant formed was dried at 100°C, and the water solubility (WS) was calculated as follows:

$$\text{solubility degree} = \frac{2 \times \text{grams of solids in the supernatant} \times 100}{\text{grams of sample}} \quad (1)$$

The water-holding capacity (WHC) of the sample was analyzed following the previously published method (Zi-Ni *et al.*, 2015). Approximately 10 mL of distilled water was added to 1 g of sample, vortexed at 25°C for 1 min, and centrifuged at 2200 g for 30 min. The supernatant was removed and the sediment was weighed to calculate WHC as follows:

$$\text{WHC (\%)} = \frac{\text{weight of wet sediment(g)} - \text{weight of the sample used(g)} \times 100}{\text{weight of the sample used(g)}} \quad (2)$$

Swelling power analysis was performed based on the method described in previous studies (Chan *et al.*, 2010; Shah *et al.* 2016). In a centrifuge tube of 50 ml, 100 mg starch was mixed with 10 ml distilled water, and heated in a water bath shaker at 90°C for 30 minutes, which was centrifuged at 2330 g for 15 minutes. The supernatant was carefully removed and the swollen wet starch sediment was weighed. The swelling power was calculated as follows:

$$\text{swelling power (\%)} = \frac{\text{weight of wet sediment(g)} \times 100}{\text{weight of the sample used(g)}} \quad (3)$$

This study used a completely randomized design, while the data obtained were analyzed by analysis of variance (ANOVA) using the Statistical Analysis System (SAS) version 9.4, at a significance level of 5% ($p \leq 0.05$). Subsequently,

Duncan's Multiple Range Test (DMRT) was performed and data were presented as means of triplicates $\pm$ standard deviation.

Results and Discussion

Physicochemical Characteristics

The results of chemical properties analysis using NS and Cilacap breadfruit RS3 at 100°C/24 h, 100°C/48 h, 121°C/24 h, 121°C/48 h, 140°C/24 h, and 140°C/48 hours are presented in Table 1.

Table 1. Chemical characteristics of Cilacap breadfruit RS3

Sample	Components		
	Amylose (%)	Amylopectin (%)	RS (%)
NS	31.01 $\pm$ 0.24 ^d	51.84 $\pm$ 0.19 ^a	10.98 $\pm$ 0.07 ^f
100°C/24 h	34.40 $\pm$ 1.28 ^c	45.07 $\pm$ 1.29 ^b	11.03 $\pm$ 0.06 ^f
100°C/48 h	35.39 $\pm$ 0.90 ^b	45.05 $\pm$ 0.77 ^b	11.88 $\pm$ 0.17 ^e
121°C/24 h	29.39 $\pm$ 0.14 ^e	38.43 $\pm$ 0.03 ^e	12.92 $\pm$ 0.05 ^d
121°C/48 h	28.48 $\pm$ 0.21 ^e	38.51 $\pm$ 0.53 ^e	14.14 $\pm$ 0.19 ^c
140°C/24 h	35.56 $\pm$ 1.09 ^b	44.80 $\pm$ 0.14 ^c	18.51 $\pm$ 0.11 ^b
140°C/48 h	37.91 $\pm$ 0.43 ^a	43.88 $\pm$ 0.74 ^d	19.41 $\pm$ 0.05 ^a

Values in the same column followed by different letters are different significantly between samples ($p \leq 0.05$). NS : Native starch; RS: Resistant Starch.

Breadfruit serves as a source of carbohydrates, with starch being the main constituent, consisting of two molecules, namely amylose, and amylopectin. The total properties of starch are determined by the amylose-amylopectin ratio (Ai *et al.*, 2013a). Amylose content in starchy food is classified into 4 groups based on amylose-to-amylopectin ratio, namely very low (2-9%), low (10-20%), intermediate (20-25%), and high (>25%) (Faridah *et al.*, 2013; Jeevetha *et al.*, 2014; Chrungoo and Devi, 2015). In this study, the native starch breadfruit contains a high amylose content of 31.01%, as it exceeded the 25% threshold. This was consistent with the 30.18% reported by (Wang *et al.*, 2011), but higher than the results of (Tan *et al.*, 2017) at 16.83%. A lower amylopectin content was obtained at 51.84%, indicating the impact of variance in cultivars, climate, soil conditions, and harvest age (Akanbi *et al.*, 2009; Faridah *et al.*, 2013; Tan *et al.*, 2017). Previous studies on breadfruit from Ile-Ife, Osun State Nigeria, reported a carbohydrate content of 84.48% (Adebowale *et al.*, 2005); and 86.48% (Akanbi *et al.*, 2009). Meanwhile, breadfruit from Ijokodo, Ibadan, Nigeria had a carbohydrate content of 86.67% (Nwokocha and Williams, 2011). Breadfruit from Cilacap, Indonesia, has a higher carbohydrate content than the 91.40% reported by

the previous research (Rahayu *et al.*, 2021). This high value was contributed by the elevated starch content of 82.85% (Rahayu *et al.*, 2021), which is the main constituent of carbohydrates (Kazeem *et al.*, 2018). This value was higher than other Indonesian breadfruit reported by Lubis *et al.* (2006), which was 57.80%. Moreover, the high carbohydrate content is essential information for the development of Cilacap breadfruit as another alternative carbohydrate source from wheat and rice (Widowati, 2009), bananas, cassava, potatoes (Lubis *et al.*, 2006), and corn. This information is also needed for the wider use of Cilacap breadfruit starch in the pharmaceutical and food industries as an ingredient (Famurewa *et al.*, 2016; Nwokocha and Williams, 2011).

Cilacap breadfruit production used the autoclaving method at a higher temperature (140°C), cold storage (4°C) longer period (48 hours), and hydrolysis in 0.1 M HCl had an effect on increasing amylose content and decreasing amylopectin content.

The increase in amylose content was caused by several factors. These included the gelatinization process during high-temperature cooking, which resulted in an extraordinary swelling of the starch granules, causing amylose molecules to come out of the granules. This process is irreversible (Dundar and Gocmen, 2013; Setiarto *et al.*, 2015), increasing amylose content. Autoclaving at high temperatures above the gelatinization threshold causes the breaking of hydrogen bonds within the double helix structure of amylopectin molecules. This process leads to a decrease in amylopectin and an increase in amylose levels (Fuentes-Zaragoza *et al.*, 2010; Setiarto *et al.*, 2015).

A retrogradation process also occurs due to the cooling process at 4°C. This process causes starch constituent molecules (amylose and amylopectin) to recombine through hydrogen bonds, forming a stable double helix structure on the surface of the granule, resulting in the development of a robust and rigid layer (Sugiyono *et al.*, 2009). The retrogradation effect causes amylopectin-amylopectin, amylopectin-amylose, and amylose-amylose molecules to recombine (Reddy *et al.*, 2014), thereby increasing amylose and decreasing amylopectin levels.

Incubation of retrograded starch in 0.1 M HCl solution for 24 hours led to the hydrolysis of Cilacap breadfruit starch, particularly affecting the amorphous part of amylopectin molecules at the branching point with α -D 1-6 linkage. This process resulted in the production of linear molecules, specifically short-chain amylose, leading to increased short-fraction amylose molecules and decreased amylopectin levels (Faridah *et al.*, 2013;

Reddy *et al.*, 2014). The production of RS3 from Cilacap breadfruit native starch by autoclaving method at 140°C, cooling at 4°C for 48 hours, and incubation in 0.1 M HCl solution for 24 hours led to an increase in the amylose content. A previous study on native breadfruit starch had a carbohydrate content of 84.48%. After passing through the starch modification process using the Heat Moisture Treatment (HMT) method (heating at 100°C for 16 hours, 18% humidity), there was a decrease in carbohydrates (Adebowale *et al.*, 2005). This indicated a decrease in starch content, followed by changes in the levels of amylose and amylopectin when the parameters were measured. High amylose content made Cilacap breadfruit meet the requirements for RS production, as one of the factors influencing and accelerating the formation process (Sugiyono *et al.*, 2009; Ozturk *et al.*, 2011; Faridah *et al.*, 2013; Jeevetha *et al.*, 2014; Setiarto *et al.*, 2015).

The RS analysis from the native starch and Cilacap breadfruit RS3 is shown in Table 1. The results showed that RS of native starch contained 10.98%. Based on the results, RS content in food ingredients is classified into 5, namely negligible (<1%), low (1-2.5%), intermediate (2.5-5.0%), high (5.0-15%), and very high (>15%) (Goñi *et al.*, 1996; Aparicio-Saguilán *et al.*, 2005). Cilacap breadfruit contains RS of 10.98% and a relatively high amylose content at 31.01%. RS levels correlate with amylose levels due to a positive between the two parameters (Khan *et al.*, 2019; Sugiyono *et al.*, 2009; Haralampu, 2000). In this study, the RS3 121°C/24 h and 121°C/48 h samples had lower amylose levels compared to others. This variation occurred due to differences in harvest time, as previously obtained by (Akanbi *et al.*, 2009; Tan *et al.*, 2017). However, the RS3 121°C/24 h and 121°C/48 h samples still had relatively high amylose levels, so the RS levels increased. These high values were attributed to autoclaving treatment at a higher temperature, which was continued with longer cold storage than NS, RS3 100°C/24 h, and 100°C/48. Additionally, high amylose starch requires pressure cooking at high temperatures (Ozturk *et al.*, 2011).

After RS3 production, the autoclaving-cooling and storage method as well as strong acids hydrolysis increased amylose content from 31.01% to 28.48-37.91% and RS content from 10.98% in native starch to 11.03-19.41%. However, a reduction was observed in amylopectin content from 51.84% in native starch to 38.51%-48.43%. The formation of RS through autoclaving-cooling continued with strong acids hydrolysis occurring in the retrogradation and hydrolysis stages. When this was conducted at different temperatures of 100, 121,

and 140°C, it allowed native starch gelatinization, leading to the damage of the granule structure due to heating. The increase in RS by this method has been reported on amylotype corn starch high amylose, Hylon V (HV), Hylon VII (HVII) (Ozturk *et al.*, 2011), Hylon VII (HVII) (Dundar and Gocmen, 2013), and arrowroot starch (Faridah *et al.*, 2013). The granule swelled irreversibly after releasing the amylose fraction (Ozturk *et al.*, 2011; Dundar and Gocmen, 2013; Setiarto *et al.*, 2015), which increased amylose content. The heating process of starch suspension by autoclaving above the gelatinization temperature led to hydrogen bonds dissociation from the amylopectin molecule double helix structure, followed by the crystalline portion removal and linear molecules release from the amylopectin (Setiarto *et al.*, 2015).

The cooling at 4°C after autoclaving caused a retrogradation process (Sugiyono *et al.*, 2009), where the amylose fraction was tied back through hydrogen bonds in the recrystallization condition, followed by re-formation of the double helix structure, and RS3 (Setiarto *et al.*, 2015). The repeated autoclaving-cooling process up to three times led to increased recombination of amylose, amylose-amylopectin, and amylopectin-amylopectin (Reddy *et al.*, 2014). Furthermore, this process contributed to high RS levels (Aparicio-Saguilán *et al.*, 2005; Ashwar *et al.*, 2016; Sugiyono *et al.*, 2009).

Treatment with strong acid is more effective in hydrolyzing gelatinized and retrograde starch compared to native starch. This treatment is usually used in the production of RS3 to increase native starch RS content (Dundar and Gocmen, 2013; Faridah *et al.*, 2013; Ozturk *et al.*, 2011). During the acid treatment, starch passes through hydrolysis, allowing amylopectin molecules to hydrolyze and form amylose molecules' short fraction (Dundar and Gocmen, 2013). Starch hydrolysis with acid is safer in breaking the amylopectin chain at the amorphous branching point compared to the crystalline portion of the amylopectin molecule (Faridah *et al.*, 2013). This also occurs in elephant foot yam starch (*Amorphophallus paeoniifolius*), which is made into RS3 through gelatinization by autoclaving at 121°C for 30 minutes and retrogradation process by cooling at 4°C for 24 h, followed by hydrolysis with the pullulanase enzyme. Furthermore, the acid treatment produced RS3 with a significant increase in resistant starch content from 24.21% to 50.18%. The pullulanase enzyme acted at the amylopectin branching point by cutting α linkage and producing amylose molecules with shorter linear chains. The result obtained was an increase in short amylose fraction and decreased amylopectin content. The linear chain of the amylose molecules' short fraction

in the retrogradation process forms a very strong gel network representing RS formation (Reddy *et al.*, 2014). The mechanism of enzyme hydrolysis was the same as acid hydrolysis in this study. Furthermore, the RS content was increased by the autoclaving-cooling method repeatedly combined with acid hydrolysis, a widely used technique for RS3 formation (Kusnandar *et al.*, 2015).

The RS content of Cilacap breadfruit starch increased with higher autoclaving temperature, ranging from 10.98% to 11.03-19.41%. Based on the results, the highest RS content of 19.41% was obtained in a sample of RS at 140°C and 48 h, which was included in the very high classification (>15%) (Goñi *et al.*, 1996).

Additional information related to the production of RS3 from Cilacap breadfruit using the autoclaving-cooling-storing method is the increase in antioxidant activity contained in the material. The antioxidant activity increased from 3.59% in native starch to 7.09-9.11% after the production of RS3. The highest antioxidant content was obtained in autoclaving at 140°C cold storage at 4°C for 48 hours at 9.11%. The method used increased the total dietary fiber of native starch from 2.73% to approximately 5.12-7.23% (Rahayu *et al.*, 2021). This increase in levels of RS, antioxidants, and dietary fiber positively influenced the production of RS3 breadfruit Cilacap. This indicated that Cilacap breadfruit from Indonesia played a significant role in the development of functional foods beneficial to health (Reddy *et al.*, 2013).

Pasting Properties

The measurements of pasting properties for Cilacap breadfruit native starch and RS3 using Rapid Visco Analyzer (RVA) are presented in

Table 2, and the viscoamylograph of RVA is shown in Figure 1.

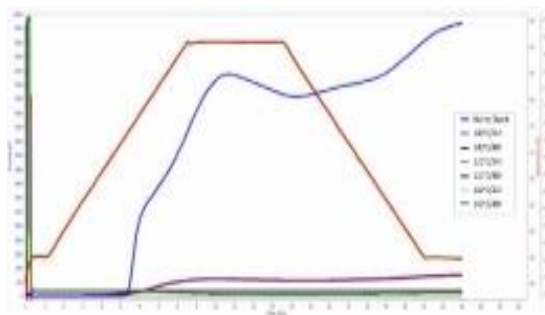


Figure 1. Viscoamylograph of NS (native starch) and Cilacap breadfruit RS3

Based on the results, the native starch pasting properties were significantly different from RS3, namely 100, 121, and 140°C, with 24 and 48 hours of storage ($p \leq 0.05$).

Generally, all the pasting properties parameters of breadfruit native starch were initially the highest but decreased after RS3 production. These parameters included breakdown, peak, trough, final, and setback viscosity, as well as peak time, and pasting temperature. The peak viscosity values of all RS3 ranged from 23.00-626.00 cP, but the breakdown viscosity values decreased significantly from 67.00 to 6.00 cP. The starch paste properties with high peak viscosity experienced a sharp decrease in breakdown viscosity value. This suggested that the starch was less resistant during processing with heat (Rahmiati *et al.*, 2017).

Table 2. Pasting properties of Cilacap breadfruit resistant starch type 3

Sample	PV (cP)	TV (cP)	BV (cP)	FV (cP)	SV (cP)	PTm (minutes)	PT (°C)
NS	7894.00±1.26 ^a	7080.00±1.13 ^a	814.00±1.02 ^a	9693.00±1.45 ^a	2613.00±0.31 ^a	10.67±0.26 ^a	72.95±0.2 ^a
100°C/24 h	625.00±1.19 ^b	558.00±0.52 ^b	67.00±0.06 ^{bc}	750.00±0.52 ^b	192.00±0.07 ^b	9.60±0.23 ^b	Nd
100°C/48 h	626.00±0.32 ^b	578.00±0.38 ^b	48.00±0.05 ^c	779.00±0.35 ^b	201.00±0.08 ^b	10.20±0.25 ^b	Nd
121°C/24 h	213.00±0.76 ^c	94.00±0.27 ^c	119.00±0.4 ^b	143.00±0.14 ^c	49.00±0.03 ^c	5.80±0.01 ^c	Nd
121°C/48 h	199.00±0.61 ^c	89.00±0.21 ^c	110.00±0.6 ^b	135.00±0.11 ^c	46.00±0.03 ^c	5.80±0.01 ^c	Nd
140°C/24 h	26.00±0.14 ^d	20.00±0.04 ^d	6.00±0.01 ^d	27.00±0.05 ^d	7.00±0.01 ^d	4.80±0.00 ^d	Nd
140°C/48 h	23.00±0.17 ^d	17.00±0.02 ^d	6.00±0.03 ^d	21.00±0.06 ^d	4.00±0.01 ^d	3.67±0.00 ^d	Nd

Values followed by different letters in the same column were significantly different between the samples ($p \leq 0.05$). Native starch, PV: Peak viscosity, TV: Trough viscosity, BV: Breakdown viscosity, FV: Final viscosity, SV: Setback viscosity, PTm: Peak time, PT: Pasting temperature, Nd: non detecte.

A study on breadfruit flour indicated high peak viscosity when measured using the Brabender Amylograph, with results exceeding 1000 BU compared to wheat flour. This high peak enabled breadfruit flour to have higher swelling power compared to wheat flour (Widowati, 2009). Native starch has the highest setback viscosity of 2613.00 cP, followed by RS3 at 100°C/24 h, 100°C/48 h, 121°C/24 h, 121°C/48 h, 140°C/24 h, and 140°C/48 h. High regressing viscosity values indicate a tendency to be more susceptible to retrogradation and syneresis, leading to easier gel formation when cooled (Rahmiati *et al.*, 2017). The properties of starch paste are crucial in the application of RS3 in the starchy food industry (Reddy *et al.*, 2014). Compared to RS3 breadfruit processed through heat moisture treatment (HMT) with various moisture (Tan *et al.*, 2017), there are differences in the pasting properties value. This is due to variations in analytical methods, tools, botanical sources, interactions between molecules, starch sample numbers (Reddy *et al.*, 2014), and changes in amylose levels (Ai, 2013a). In this study, the pasting temperature of native starch formed at 73.30°C, which was lower compared to the 84.05°C obtained by Akanbi *et al.* (2009). However, the value was higher than the 66.60°C reported by Adebawale *et al.* (2005), with breadfruit starch samples from Ile-Ife, Osun State, Nigeria. The pasting temperature in this study was not detected for all RS3 produced due to the destruction of starch granules during high-temperature cooking (100, 121, and 140°C). This phenomenon was confirmed by previous investigations on red beans and elephant foot yam (Reddy *et al.*, 2013; Reddy *et al.*, 2014).

Thermal Properties

The gelatinization parameters of the onset (To), peak (Tp), and conclusion (Tc) temperature

indicating the thermal properties are presented in Table 3. The DSC showed the enthalpy transition analysis results and transition temperature associated with the melting of starch crystalline parts during heating. Cilacap breadfruit starch compared to all the RS3 products showed an increase in To, Tp, Tc, and gelatinization temperature range (Tc-To) values, but a decrease in gelatinization enthalpy (ΔH). To is the initial temperature for gelatinization when the starch granules lose their polarity properties. Meanwhile, Tc is the final gelatinization temperature when 98% polarized starch granules are lost, and Tp is the peak gelatinization temperature of the starch (Wang and Copeland, 2013).

The starch granule crystalline portion has a melting temperature (To, Tp, and Tc), which is indirectly controlled by the surrounding amorphous portion (Gunaratne and Hoover, 2002). In this study, the Tp value of RS3 increased compared to native starch. This indicated that the starch granules were broken due to hydrolysis during autoclaving and acid treatment, leading to more crystalline compared to amorphous areas (Alonso-Gomez *et al.*, 2016). Consequently, the RS3 crystalline melting process required higher temperatures (Tan *et al.*, 2017). After the autoclaving-cooling process, 24 and 48 hours storage and strong acid hydrolysis continued, resulting in higher Tc-To due to the starch granule structural rearrangement to become stable and regular (Tan *et al.*, 2017). The value of ΔH RS3 decreased compared to native starch. This was due to a decrease in a disturbance in the crystalline and non-crystalline region by the double helix structure because of heat treatment (100, 121, and 140°C). This was followed by strong acids hydrolysis, ensuring a decrease in the energy required to cut off the double helix bond (Garcia *et al.*, 2016). The pattern of native starch and RS3

Table 3. Thermal properties of Cilacap breadfruit resistant starch type 3

Sample	To (°C)	Tp (°C)	Tc (°C)	(Tc-To) (°C)	ΔH (J/kg)
NS	145.25±2.48 ^a	150.75±0.25 ^c	155.68±2.79 ^c	10.43±0.31 ^c	-3.46±1.71 ^b
100°C/24 h	156.02±8.52 ^a	161.34±7.12 ^{ab}	166.26±5.40 ^{ab}	10.24±3.12 ^{bc}	-2.61±0.75 ^c
100°C/48 h	152.43±0.27 ^a	160.13±2.92 ^{ab}	167.46±5.42 ^{ab}	15.03±5.15 ^{bc}	-4.00±1.36 ^c
121°C/24 h	133.71±24.56 ^b	156.69±32.19 ^b	161.24±28.78 ^b	27.53±4.22 ^a	-6.92±2.95 ^b
121°C/48 h	140.73±0.75 ^a	163.65±0.47 ^a	168.10±1.39 ^{ab}	27.37±0.64 ^a	-5.25±1.17 ^a
140°C/24 h	132.00±3.97 ^{ab}	155.45±8.87 ^b	160.02±9.15 ^b	28.02±5.18 ^a	-6.50±3.33 ^b
140°C/48 h	154.37±9.31 ^a	164.07±5.92 ^a	171.15±1.48 ^a	16.78±7.83 ^b	-5.41±0.66 ^b

Values followed by different letters in the same column were significantly different between the samples ($p \leq 0.05$). NS: Native starch, To: Onset temperature, Tp: Peak temperature, Tc: Conclusion temperature, and ΔH : Gelatinization enthalpy.

Cilacap breadfruit thermal properties was confirmed by the previous study on Madagascar breadfruit RS3 production (Tan *et al.*, 2017).

Functional Characteristics

The functional characteristics analysis using NS and Cilacap breadfruit RS3 at 100°C/24 h, 100°C/48 h, 121°C/24 h, 121°C/48 h, 140°C/24 h, and 140°C/48 are presented in Table 4.

Table 4. Functional characteristics of Cilacap breadfruit RS3

Sample	WS (%)	WHC (%)	SP (%)
NS	3.09±0.17 ^a	256.80±0.46 ^b	1431.00±0.2 ^a
100°C/24 h	12.33±0.15 ^d	270.67±0.58 ^a	91.99±0.01 ^f
100°C/48 h	12.79±0.03 ^c	271.56±2.26 ^a	88.13±0.00 ^f
121°C/24 h	8.82±0.07 ^f	222.54±6.72 ^c	565.67±0.10 ^c
121°C/48 h	11.07±0.24 ^e	216.84±1.16 ^b	611.67±0.03 ^b
140°C/24 h	23.05±0.18 ^a	159.28±7.96 ^c	188.93±0.01 ^d
140°C/48 h	19.92±0.25 ^b	151.00±1.07 ^c	161.67±0.02 ^e

Values in the same column followed by different letters are different significantly between samples ($p \leq 0.05$). NS: Native starch, WS: Water solubility, WHC: Water holding capacity, SP: Swelling power.

Cilacap breadfruit RS3 yielded a significant result ($p \leq 0.05$), with a higher water solubility (WS) compared to native starch, indicating an improvement in solubility properties. The native starch WS value (3.09%) increased after being produced into RS3 by autoclaving-cooling and acid hydrolysis methods (8.82-23.05%). The highest solubility value (23.05%) was obtained from a sample of RS3 at 140°C and 24 hours. This was confirmed by the previous study on high-amylose corn starch RS3 production, using the autoclaving-cooling method accompanied by acid hydrolysis, samples with autoclaving at 140°C and cooling at 4°C for 24 h, producing the highest WS values of 6.32% (Dundar and Gocmen 2013). The increase in solubility due to the autoclaving-cooling process was continued by acid hydrolysis performance. This was because of amylose-amylopectin molecule degradation, which produced short-chain molecules with high solubility.

Furthermore, the formation of short-chain molecules decreased molecular weight with varying molecular structures of starch. (Reddy *et al.*, 2017). The increase in solubility also occurred in the production of breadfruit RS through the heat-moisture treatment (HMT) process. The rise in temperature of the HMT from 50-80°C, and humidity 18%, further increased its solubility from 34.47% to 49.93% (Adebawale *et al.*, 2005).

The WHC value of Cilacap breadfruit native starch was 156.80%, which increased significantly

($p \leq 0.05$) at RS3 conditions of 100°C, 24 h, and 48 h (290.67%, and 317.56%). However, this value decreased with an increase in autoclaving temperature (121 and 140°C) and 24 - 48 h cold storage. The acid hydrolysis-cooling-autoclave process caused the amylopectin chains from starch grains to partially degrade, producing chains with low molecular weight, to obtain a higher affinity with water molecules (Ratnaningsih *et al.*, 2020; Reddy *et al.*, 2017). The increase in WHC was also confirmed in RS3 production through autoclaving-cooling of rice starch (Ashwar *et al.*, 2016). The production of RS3 Cilacap breadfruit in autoclaving-cooling continued with acid hydrolysis at 100°C showed a higher WHC value compared to the autoclaving treatment at 121 and 140°C due to the amylose-amylopectin ratio. RS3 autoclaving at 100°C, amylopectin contents were still higher than RS3 autoclaving at 121 and 140°C. This amylopectin molecule was amorphous or rift due to its more branching points at α -D 1-6 bonds (Ai *et al.*, 2013b). Due to their characteristic structure, these molecules were susceptible to water infiltration, resulting in higher wettability compared to amylose molecules (Rahmiati *et al.*, 2017). Additionally, autoclaving at 100°C was not intensive enough to break down the amorphous part of amylopectin molecules, leading to a higher WHC value. In the production of RS3 Cilacap breadfruit, the storage time also reduced the WHC value. This result was different from the manufacture of RS3 using the autoclaving-colling method and strong acid hydrolysis of high amylose corn starch. However, the use of corn starch with a very high amylose content (70%) and high temperatures increased the water binding value (Dundar and Gocmen, 2013). These variations were attributed to differences in starch sources and compositions.

The highest native starch swelling power was 1431.00%, which decreased after the production of RS3. This indicated that Cilacap breadfruit native starch was very easy to cook, as the swelling represented the tenacity of the bonds on the starch granule crystalline part. The swelling power in cold and heated water also decreased when the granules possessed larger crystalline areas with stronger bonds (Reddy *et al.*, 2014). This phenomenon was also observed in the RS3 production of Cilacap breadfruit starch. Generally, the SP value decreased from 1431.00% to 91.99-611.67% compared to the native starch. The decrease was associated with increased interactions between the amylose-amylose chain and the amylose-amylopectin chain, increasing the complete crystalline portion (Ashwar *et al.*, 2016). The interaction occurred between natural amorphous with crystalline parts between the starch constituent chains (Reddy *et al.*, 2014).

Therefore, the high and low SP values were discovered to correlate with the variation in starchy amylopectin levels (Rashid *et al.*, 2020). In this study, amylopectin levels of Cilacap breadfruit starch (native starch) and RS3 were low, leading to susceptibility to swelling. A previous study on the production of RS3 breadfruit in Cilacap showed that the functional properties of the Water Absorption Index (WAI) were increasing, as the native starch WAI 0.52 g/g increased in all RS3 produced to 1.94-3.47 g/g. The measurement of Oil Holding Capacity (OHC), decreased in value from original starch OHC of 1.01 g/g to 0.59-0.79 g/g at 100 and 121°C autoclaving treatment. However, the value increased at 140°C autoclaving temperature, cooling at 4°C during 24 and 48 hours 1.80 g/g and 1.53 g/g, respectively (Rahayu *et al.*, 2021). As indicated by the way, OHC, and WHC values, Cilacap breadfruit RS3 can be applied as a thickener in puddings, sauces (Ratnaningsih *et al.*, 2020), and soups (Kazeem *et al.*, 2018), and meat products to enhance palatability and preserve taste (Ratnaningsih *et al.*, 2020). The high SP value in native breadfruit can be applied to products that require more swelling power such as noodles (Kazeem *et al.*, 2018) and bread. Meanwhile, Cilacap breadfruit RS3 applies to products with lower swelling power, including pastries.

The application of RS3 Cilacap breadfruit, with its high amylose content, RS, antioxidant, and dietary fiber, offers a promising potential as a functional food ingredient. Furthermore, it can lower blood glucose and glycemic index, preventing diabetes mellitus type 2, cancer, and obesity (Ratnaningsih *et al.*, 2020).

Conclusions

The conclusion of this research shows that Cilacap breadfruit starch has a high amylose content, namely 31.01%, making it suitable for producing RS3. The original starch produced is converted into RS3 by autoclaving at a high temperature of 140, storage time is 48 hours. This process significantly increases amylose content, RS, onset, peak, conclusion, and water solubility. However, the production process causes a decrease in all pasting properties, gelatinization enthalpy and swelling power. Native starch and RS3 of Cilacap breadfruit are included in the high amylose category. The autoclaving cooling process on Cilacap breadfruit starch has an effect on increasing RS levels, so it can be used as an alternative source of resistant starch for the development of functional food.

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References

- Ai, Y. (2013a). Structures, properties, and digestibility of resistant starch. [PhD dissertation] Ames Iowa: Iowa State University, Digital Repository @Iowa State University.
- Ai, Yongfeng, Jovin Hasjim, and Jay-lin Jane. (2013b). Effects of Lipids on Enzymatic Hydrolysis and Physical Properties of Starch. *Carb. Polym.*, 92(1):120-27.
- Adebowale, Kayode Oyeboade, Bamidele Iromidayo Olu-Owolabi, Esther Kehinde Olawumi, and Olayide Samuel Lawal. (2005). Functional Properties of Native, Physically and Chemically Modified Breadfruit (*Artocarpus altilis*) Starch. *Indust. Crops and Products* 21(3):343-51.
- Akanbi, T.O., S. Nazamid, and A.A Adebowale. (2009). Functional and Pasting Properties of a Tropical Breadfruit (*Artocarpus altilis*) Starch from Ile-Ife, Osun State, Nigeria. *Int. Food Research J.*, 16:151-57.
- Alonso-Gomez, L., Niño-López, A.M., Romero-Garzón, A.M., Pineda-Gomez, P., Real-Lopez, A.D., and Rodriguez-Garcia, M.E. (2016). Physicochemical Transformation of Cassava Starch during Fermentation for Production of Sour Starch in Colombia. *Starch/Stärke*, 68:1-9.
- Aparicio-Saguilán, A., Flores-Huicochea, E., E., Tovar, J., García-Suárez, F., Gutiérrez-Meraz, F., and Bello-Pérez, L.A. (2005). Resistant Starch-Rich Powders Prepared by Autoclaving of Native and Lintnerized Banana Starch: Partial Characterization. *Starch/Stärke*, 57:405-12.
- Ashwar, B.A., Gani, A., Shah, A., Masoodi, F.A., and Saxena, D.C. (2016). Production of Resistant Starch from Rice by Dual Autoclaving-Retrogradation Treatment: In vitro Digestibility, Thermal and Structural Characterization. *Food Hydrocol.*, 56:108-17.
- Chan, H.T., Bhat, R., and Karim, A.A. (2010). Effects of Sodium Dodecyl Sulphate and Sonication Treatment on Physicochemical Properties of Starch. *Food Chem.*, 120:703-9.
- Chungoo, N.K. and Devi, A.G. (2015). Morphological and Rheological Properties of Starches Separated from Cultivars of Rice (*Oryza sativa* L.) from North East India. *American J. of Plant Sci.*, 6:2019-31.
- Deivanai S., and Bhore. S.J. (2010). Breadfruit (*Artocarpus altilis* Fosb.) - An Underutilized and Neglected Fruit Plant Species. *Middle-East J. of Scientific Research*, 6(5):418-28.
- Dundar, A.N., and Gocmen, D. (2013). Effects of Autoclaving Temperature and Storing Time on Resistant Starch Formation and Its Functional and Physicochemical Properties. *Carb. Polym.*, 97:764-71.
- Englyst, H.N., Kingman, S.M., and Cummings J.H. (1992). Classification and Measurement of Nutritionally Important Starch Fractions. *European J. of Clin. Nutri.*, 46(Suppl.2):S33-S50.

- Famurewa, J.A.V., Pele, G.I., Esan, Y.O., and Jeremiah, B.P. (2016). Influence of Maturity and Drying Methods on the Chemical, Functional and Antioxidant Properties of Breadfruit (*Artocarpus altilis*). *British Biotech. J.*, 16(1):1-9.
- Faridah, D.N., Rahayu, W.P., and Apriyadi, M.S. (2013). Modifikasi Pati Garut (*Marantha arundinacea* L.) Dengan Perlakuan Hidrolisis Asam Dan Siklus Pemanasan-Pendinginan Untuk Menghasilkan Pati Resisten Tipe 3 [Modification of Arrowroot (*Marantha Arundinacea* L.) Starch Through Acid Hydrolysis and Autoclaving-Co. J. Teknol. Indus. Pert., 23(1):61-69.
- Fuentes-Zaragoza, E., Riquel-Navarret, M.J., Sanchez-Zapata, E., and Perez-Alvarez, J.A. (2010). Resistant Starch as a Functional Ingredient : A Review. *Food Research Int.*, 43:931-42.
- Garcia, M.C., Franco, C.M.L., Júnior, M.S.S., and Caliri, M. (2016). Structural Characteristics and Gelatinization Properties of Sour Cassava Starch. *J. of Thermal Analys. and Calorimetry.*, 123(2):919-26.
- Goñi, I., García-Diz, L., Mañas, E., and Saura-Calixto, F. (1996). Analysis of Resistant Starch: A Method for Foods and Food Products. *Food Chem.*, 56(4):445-49.
- Gunaratne, A., and Hoover, R. (2002). Effect of Heat-Moisture Treatment on the Structure and Physicochemical Properties of Tuber and Root Starches. *Carb. Polym.*, 49:425-37.
- Haralampu, S.G. (2000). Resistant starch a review of the physical properties and biological impact of RS 3. *Carb. Polym.*, 41:285-292.
- Jeevetha, S., Barakatun-Nisak, M.Y., Ngan, H.B., Ismail, A., and Azlan, A. (2014). Relationship between Amylose Content and Glycemic Index of Commonly Consumed White Rice. *IOSR J. of Agric. and Veteri. Sci.*, 7(9 Ver. III):12-18.
- Kazeem, A.B., Idowu, A.A., and Victor, F.J.A. (2018). Effect of Drying Temperature on the Chemical Qualities of Breadfruit. *Int. J. of Food Sci. and Biotech.*, 3(1):1-6.
- Khan, A., Rahman, U.U., Siddiqui, S., Irfan, M., Shah, A.A., Badshah, M., Hasan, F., and Khan, S. (2019). Preparation and Characterization of Resistant Starch Type III from Enzymatically Hydrolyzed Maize Flour. *Molec. Biol. Report.*, 46:4,585-4,580.
- Kusnandar, F., Hastuti, H.P., and Syamsir, E. (2015). Pati Resisten Sagu Hasil Proses Hidrolisis Asam Dan Autoclaving- Cooling [Resistant Starch of Sago from Acid Hydrolysis and Autoclaving-Cooling Processes]. *J. Teknol. dan Indus. Pangan*, 26(1):52-62.
- Lubis, Y.M., Kumalaningsih, S., and Susanto, T. (2006). Tepung Komposit Berbasis Tepung Sukun (*Artocarpus altilis*) Hasil Modifikasi Alkali Untuk Pembuatan Biskuit. *J. Teknol. Pert.*, 7(3):173-83.
- Nasrin, T.A.A., and Anal, A.K. (2014). Resistant Starch III from Culled Banana and Its Functional Properties in Fish Oil Emulsion. *Food Hydrocol.*, 35:403-9.
- Nwokocha, L.M, and Williams, P.A. (2011). Comparative Study of Physicochemical Properties of Breadfruit (*Artocarpus altilis*) and White Yam Starches. *Carb. Polym.*, 85:294-302.
- Ozturk, S., Koksels, H., and K.W.Ng, P. (2011). Production of Resistant Starch from Acid-Modified Amylotype Starches with Enhanced Functional Properties. *J. of Food Engin.*, 103(2):156-64.
- Praseptianga, D., Tryas, A.A., Affandi, D.R., Atmaka, W., Ariyantoro, A.R., and Minardi, S. (2018). Physical and Chemical Characterization of Composite Flour from Canna Flour (*Canna edulis*) and Lima Bean Flour (*Phaseolus lunatus*). *Proceedings of AIP Conference* 1927, 030020. The American Institute of Physics, 030020-1-030020-5.
- Rahayu, N.S., Praseptianga, D., Haryanto, B., and Samanhuji. (2019). Fruit Identification and Effect of Starch Isolation Methods on Color Attributes of Cilacap Bread Fruit's Starch. *Proceeding of IOP Conference Series: Material Sci. and Engin.* 633 012037, 1-6.
- Rahayu, N.S., Praseptianga, D., Haryanto, B., and Samanhuji. (2021). Preparation and Characterization of Type 3 Resistant Starch from Cilacap Breadfruit (*Artocarpus altilis* (Parkinson) Fosberg) Starch. *Int. J. on Advan. Sci. Engin. Inform. Tech.*, 11(4):1,623-1,632.
- Rahmianti, T.M., Purwanto, Y.A., Budijanto, S., and Khumaida, N. (2017). Sifat Fisikokimia Tepung Dari 10 Genotipe Ubi Kayu (*Manihot esculenta* Crantz) Hasil Pemuliaan (Physicochemical Properties of Cassava Flour (*Manihot esculenta* Crantz) of 10 Breeding Genotypes). *Agritech.*, 36(4):459-466.
- Rashid, R.S.A., Mohamed, A.M.D., Achudan, S.N., and Mittis, P. (2020). Physicochemical Properties of Resistant Starch Type III from Sago Starch at Different Palm Stages. *Proceedings of Materials Today*: xxx: 1-5.
- Ratnaningsih, N., Suparmo., Harmayani, E., and Marsono, Y. (2020). Physicochemical Properties, in Vitro Starch Digestibility, and Estimated Glycemic Index of Resistant Starch from Cowpea (*Vigna Unguiculata*) Starch by Autoclaving-Cooling Cycles. *Int. J. of Biologic. Macromole.*, 142:191-200.
- Reddy, C.K., Haripriya, S., Mohamed, A.N., and Suriya, M. (2014). Preparation and Characterization of Resistant Starch III from Elephant Foot Yam (*Amorphophallus paeonifolius*) Starch. *Food Chem.*, 155:38-44.
- Reddy, C.K., Suriya, M., Vidya, P.V., and Haripriya, S. (2017). Synthesis and Physico-Chemical Characterization of Modified Starches from Banana (*Musa AAB*) and Its Biological Activities in Diabetic Rats. *Int. J. of Biologic. Macromole.*, 94:500-507.
- Reddy, C.K., Suriya, M., and Haripriya, S. (2013). Physico-Chemical and Functional Properties of Resistant Starch Prepared from Red Kidney Beans (*Phaseolus vulgaris*.L) Starch by Enzymatic Method. *Carb. Polym.*, 95:220-26.
- Setiarto, R.H.B., Jenie, B.S.L., Faridah, D.N., and Saskiawan, I. (2015). Kajian Peningkatan Pati Resisten Yang Terkandung Dalam Bahan Pangan Sebagai Sumber Prebiotik [Study of Development Resistant Starch Contained in Food Ingredients as Prebiotic Source]. *J. Ilmu Pert. Indonesia.*, 20(3):191-200.
- Shah, A., Masoodi, F.A., Gani, A., and Ashwar, B.A. (2016). In-Vitro Digestibility, Rheology, Structure, and Functionality of RS3 from Oat Starch. *Food Chem.*, 212:749-58.
- Singh, J. and Singh, N. (2003). Studies on the Morphological and Rheological Properties of Granular Cold Water Soluble Corn and Potato Starches. *Food Hydrocol.*, 17:63-72.
- Sugiyono, Pratiwi, R., and Faridah, D.N. (2009). Modifikasi Pati Garut (*Marantha arundinacea*) Dengan Perlakuan Siklus Pemanasan Suhu Tinggi-Pendinginan (Autoclaving-Cooling-Cycling) Untuk Menghasilkan Pati Resisten Tipe III [Arrowroot (*Marantha arundinacea*) Starch Modification Through Autoclaving-Cooling Cycling Treatment to Produce Resistant Starch Type III]. *J. Teknol. dan Indus. Pangan*, (1):17-24.
- Tan, X., Li, X., Chen, L., Xie, F., Li, L., and Huang, J. (2017). Effect of Heat-Moisture Treatment on Multi-Scale Structures and Physicochemical Properties of Breadfruit Starch. *Carb. Polym.*, 161:1-37.
- Wang, S., and Copeland, L. (2013). Molecular Disassembly of Starch Granules during Gelatinization and Its Effect on Starch Digestibility: A Review. *Food and Function.*, 4:1,564-80.

- Wang, X., Chen, L., Li, X., Xie, F., Liu, H., and Yu, L. (2011). Thermal and Rheological Properties of Breadfruit Starch. *J. of Food Sci.*, 76(1):E55-61
- Widowati, S. (2009). Prospek Sukun (*Artocarpus communis*) Sebagai Pangan Sumber Karbohidrat Dalam Mendukung Diversifikasi Konsumsi Pangan. *PANGAN*, XVIII(56):67-75.
- Williams, P.C., Kuzina, F.D., and Hylanka, I. (1970). A Rapid Colorimetric Procedure for Estimating the Amylose Content of Starches and Flours. *Cereal Chem.*, 47(288):411-20
- Zi-Ni, T., Rosma, A., Karim, A.A., and Liong, M.T. (2015). Functional Properties of Resistant Starch Type-III from Metroxylon Sagu as Affected by Processing Conditions. *Pertanika J. Trop. Agricul. Sci.*, 38(3):399-412.