Research Article

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Characteristics of RS3 Starch Gembolo (*Dioscorea bulbifera* bulbils) with Several Autoclaving-Cooling Cycles

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ABSTRACT: Gembolo (*Dioscorea bulbifera* bulbils) is a tuber that can be used as an alternative carbohydrate source. This tuber contains various essential minerals and vitamins; it also has a starch content of 6.627% and an amylose content of 30.63%. Research into gembolo has been limited to its physical and chemical characterization and its nutritional content. Using gembolo starch as a source of type RS3 via the autoclaving-cooling method has not yet been studied. RS3 promotes digestive and metabolic health by functioning as a type of fiber. Using gembolo starch as a source of type RS3 via the autoclaving-cooling method has not yet been studied. RS3 promotes digestive and metabolic health by functioning as a type of fiber. The cycle frequency used consisted of 1 to 5 autoclaving-cooling cycles. The analysis of the physicochemical properties included the following: RS, starch, amylose, amylopectin, water content, granule morphology, pasting properties, and water-holding capacity (WHC) and oil-holding capacity (OHC). After modification of gembolo starch with 1-5 autoclaving-cooling cycles, there was a 4.49% decrease in water content and a 9.65% decrease in starch content. There was also a 8.56% decrease in amylose content and a 33.29% increase in RS content. An increase in WHC of 144.22%, an increase in OHC of 2.91%, and a decrease in the pasting profile of 7753 cP. Based on these data, the treatment of 5 autoclaving-cooling cycles produced the highest RS of 74%. From this research, gembolo can be a potential source of functional RS3 starch.

Keywords: amylopectin, amylose, color, pasting profile, SEM

INTRODUCTION

One variety of tuber that may be used as a substitute source of carbohydrates is gembolo (*Dioscorea bulbifera* bulbils). Important minerals, including calcium, magnesium, salt, vitamin B1, B3, vitamin C, and protein, may be found in this tuber (Ezeabara & Anona, 2018). Gembolo also contains type-C starch crystal properties (Jiang *et al*., 2012), with an amylose concentration of 30.63% and an amylopectin content of 69.37% (Correa, Pérez, & Villegas, 2014). Gembolo has a comparatively high fiber content of 31.80 grams (Sintianingrum, 2013) and a total starch content of 62.7% (Jiang *et al*., 2012), making it a viable option for creating functional food components.

Research on gembolo has largely focused on its physical characterization and nutritional content. Gembolo starch is used as a source of resistant starch type 3 (RS3) through the autoclaving-cooling method, which has not yet been conducted. RS3 is a form of resistant starch formed through repeated thermal processing (heating and cooling), which causes starch retrogradation. This method is simple, economical, and safe as it does not use additional chemicals.

Digestive enzymes cannot break down resistant starch (RS) in the small intestine. Instead, the large intestine's probiotic bacteria ferment it (Bimo Setiarto *et al*., 2015). RS3 plays a critical role in regulating the body's metabolism and preserving intestinal health. These advantages imply that RS3 may be a useful food ingredient for avoiding degenerative illnesses such as diabetes mellitus, obesity, cancer, and heart disease.

Several methods for producing RS3 namely chemical, enzymatic, and physical. The physical method was used in this study because it is safer and more natural, does not use hazardous chemicals, is relatively inexpensive, and is easy to apply. The physical method used was autoclaving-cooling.

The autoclaving-cooling process is carried out by suspending starch in water, heating it in an autoclave at a high temperature to trigger gelatinization, and then cooling it to form a retrograded starch structure (Bimo Setiarto *et al*., 2015). Gelatinization causes starch granules to expand, increasing viscosity. During cooling,

recrystallization of amylose and amylopectin molecules occurs, resulting in a double helix structure due to hydrogen bonds between short amylose chains. This structure then forms crystallites through the recrystallization (Haralampu, 2000).

Several factors that influence the formation of RS3 through the autoclaving-cooling method include starch crystallinity, granule structure, starch source, amylose and amylopectin content, retrogradation level, number of processing cycles, interaction with dietary fiber, and the drying method used (Sajilata, Singhal, & Kulkarni, 2006).

Based on the above, research on the characteristics of type 3 resistant starch from gembolo starch with varying numbers of autoclaving-cooling cycles is necessary. The results of this research are expected to develop the potential of gembolo as a source of type 3 resistant starch beneficial for both food and non-food applications.

MATERIALS AND METHODS

Research Materials and Equipment

Materials

The materials used to make RS3 were gembolo from Malang, water, NaCl, while the materials used for analysis were distilled water, palm oil, H_2SO_4, ethanol, 0.1 N NaOH, HCl-KCl buffer pH 1.5, pepsin (Merck Inc, Germany), Tris-maleate buffer, α-amylase (Sigma A3176, Sigma-Aldrich Inc, US), amyloglucosidase (sigma A9913, Sigma-Aldrich Inc, US), glucose oxidase-peroxidase reagent kit (K-GLUC, Megazyme Inc, Ireland) and iodine solution.

Equipment

Cabinet dryer, blender, 100-mesh sieve, polyethylene, autoclave, refrigerator, spatula. Equipment used in analysis Chromamometer CR-400/410, dish, 50 mL centrifuge tube, 100 mL measuring flask, 25 mL graduated flask, vortex, petri dish, oven, weighing bottle, desiccator, reaction tube, water bath shaker, spectrophotometer, Scanning Electron Microscopy (SEM) (FEI, Inspect TM S50, USA) and Rapid Visco Analyzer TECMASTER 2061904 TMA (RVA).

Research Location and Time

The research was conducted from August 2023 to March 2025 at the biotechnology, food nutrition, incubator, and engineering laboratories of the Faculty of Agricultural Technology, Gadjah Mada University.

Research Stages

Extraction of Gembolo Starch

According to Kusnandar, Mutmainah, and Muhandri (2021), the starch extraction procedure involves peeling, cleaning, cutting the gembolo into pieces, and soaking it in a 15% NaCl solution for 15 minutes to remove the mucilage. The gembolo is rinsed under running water until clean, then grated. To extract gembolo starch, separate the starch suspension and pulp, re-extract, sediment for 13 hours, dry in a cabinet dryer at 50 °C for 3 hours, grind, and sift through a 60-mesh sieve. Natural gembolo starch was tested for RS, starch, amylose, amylopectin, and moisture content. Starch granules (SEM), pasting characteristics, WHC, OHC, starch color, and yield are among the physical features investigated.

Production of RS3 gembolo with several autoclaving-cooling cycles

The production of RS3 gembolo is cited in the studies of Sugiyono *et al*. (2009) and Ratnaningsih *et al*. (2020). Twenty grams of gembolo starch is combined with eighty milliliters of purified water with a hot plate stirrer. The resulting starch paste was autoclaved at 121 °C for 15 minutes, thereafter cooled to ambient temperature for one hour, and stored at 4 °C for 24 hours (cycles 1 through 5). The material was further dried in a cabinet drier at 60 °C for 24 hours, then passed through a 100-mesh sieve to yield RS3 gembolo. Utilizing a 100-mesh filter for the production of RS3 gembolo. The chemical analysis of RS3 gembolo encompasses starch content, amylose, amylopectin, and moisture content. Conversely, physical analysis encompasses starch granules (SEM), pasting properties, water-holding capacity (WHC), oil-holding capacity (OHC), starch color, and yield.

Analysis of natural gembolo starch and RS3 gembolo

The following are some analyses of natural gembolo starch and RS3 gembolo using physical and chemical property analysis:

Chemical property analysis

RS content (Goni et al. 1996)

The resistant starch (RS) content of the samples was determined using the direct technique established by Goni *et al*. (1996). A solution containing 20 mg of pepsin (Merck No. 7190, 2000 FIT-μ/g) was used to incubate 100 mg of pulverized material at 40 °C for 60 minutes to remove any protein. A tris-maleate solution was formulated, including 40 mg of pancreatic α-amylase (Sigma No. A-3176, 23 IU/mg). The solution was subsequently combined with a digestible starch (DS)

solution and incubated at 37 °C for 16 hours. The hydrolysates were centrifuged, and the residues were solubilized and treated with amyloglucosidase (Boehringer No. 102857) at 60 °C for 45 minutes to hydrolyze the resistant starch. A glucose oxidase-peroxidase kit (Sigma No. G3660) was utilized to quantify glucose levels, and RS, consisting of fractions of RS types I, II, and III, was determined as mg of glucose multiplied by 0.9. In accordance with the revised protocol for total starch quantification by Goni *et al*. (1997), 50 mg of pulverized samples were suspended in 6 ml of 2M KOH and incubated for 30 minutes at ambient temperature. The subsequent stage involved hydrolyzing the solubilized starch. The procedure involved adding 60 µl of amyloglucosidase, followed by incubation at 60 °C for 45 minutes in a shaking water bath. The glucose concentration in the sample was quantified using a glucose oxidase-peroxidase kit after centrifugation (15 minutes at 4500 g). The total starch content was determined using the formula: mg of glucose × 0.9. The digestible starch (DS) was estimated by subtracting resistant starch (RS) or indigestible starch from the total starch. This was articulated as a proportion of the sample mass.

$$\text{RS content} = \frac{x \times fp \times 0.9}{\text{sample weight (mg)}}$$

Starch content using the acid hydrolysis method (AOAC, 1995)

a. Preparation of standard curve

Dilutions were executed at concentrations of 2, 4, 6, 8, and 10 mg per 100 ml. Initially, 1 mL of normal glucose solution was obtained. Subsequently, one tube was filled with 1 mL of distilled water to serve as a blank. Then, 1 mL of Nelson's reagent was introduced and the solution was left to incubate in a water bath for 20 minutes. After chilling the solution, 1 ml of arsenomolybdate reagent was poured in, and the mixture was vortexed until all the $CuSO_4$ precipitates had dissolved completely. Then, 7 mL of distilled water was added and the solution was agitated until uniform. The absorbance level of each solution was measured at a wavelength of 540 nm.

b. Sample measurement

The sample (flour) was weighed at 2.5 g, transferred to an Erlenmeyer flask, and then 250 mL of distilled water was added and mixed. Twenty mL of 30% hydrochloric acid was introduced. The sample was heated using a reflux condenser for 2.5 hours. After heating, it was allowed to cool. Once cooled, it was neutralized with a 40% NaOH solution and diluted to a final volume of 500 mL, then filtered through filter paper. A 1:100 dilution of the starch solution was prepared by combining 1 mL of the solution with 9 mL of distilled water. The mixture was vortexed, and then 1 mL of solution and 9 mL of distilled water were added and vortexed once more. Subsequently, 1 mL of the solution was combined with 1 mL of Nelson-Somogy reagent. The mixture was heated in water for 20 minutes, then allowed to cool. After cooling, 1 mL of distilled water and 1 mL of arsenomolybdate were added, mixed thoroughly, and the extinction coefficient was measured at a wavelength of 540 nm. The starch content may be determined by the formula:

$$\text{Starch content (\%bb)} = \frac{x\left(\frac{\text{mg}}{\text{ml}}\right) \times \text{sample volume (ml)} \times \text{dilution factor}}{\text{initial sample weight(g)}} \times 0.9 \times 100\%$$

$$\text{Starch content (\%bk)} = \frac{\text{starch content (bb)}}{1 - \text{water content}} \times 100\%$$

Amylose content (Juliano, 1971)

a. Preparation of standard curve

Initially, 0.04 g of pure amylose was deposited in a test tube. Subsequently, 1 mL of 95% ethanol and 9 mL of 1 N NaOH were added, and the resultant liquid was heated for 10 minutes. A 100-mL volumetric flask was utilized to hold the standard solution, which was then added to it. Distilled water was added to the flask until it reached the specified level. Five milliliters of the standard solution were transferred into a 100-mL volumetric flask, accompanied by 1, 2, 3, and 4 milliliters. Subsequently, we included 0.2, 0.4, 0.6, 0.8, and 1 mL of 1 N acetic acid. Subsequently, 2 mL of iodine solution was applied to the designated spot. Subsequently, 2 mL of distilled water was incorporated. Subsequently, the solution was allowed to rest for 20 minutes, after which the absorbance was measured at 625 nm.

b. Sample measurement

A 100 mg specimen was introduced into a test tube. Subsequently, 1 mL of 95% ethanol and 9 mL of 1 N NaOH were included. The mixture was subjected to heat for 10 minutes. The sample was then transferred to a 100 mL volumetric flask, and distilled water was added to the calibration mark. A 5 mL aliquot was subsequently transferred to a 100 mL volumetric flask. Following this, 1 mL of 1 N acetic acid and 2 mL of iodine solution were added. Distilled water was thereafter added to the designated mark. Following a 20-minute incubation at ambient temperature, absorbance at 625 nm was

measured by spectrophotometry, and the amylose concentration was determined from the standard curve.

$$\text{Amylose Content (\%bb)} = \frac{x\left(\frac{\text{mg}}{\text{ml}}\right)\times\text{sample volume (ml)}\times\text{dilution factor}}{\text{initial sample weight (g)}} \times 0.9 \times 100\%$$

$$\text{Amylose content (\%bk)} = \frac{\text{amylose content (bb)}}{100 - \text{moisture content}} \times 100\%$$

Amylopectin content

Amylopectin content is calculated using the following formula:

$$\text{Amylopectin content (\%bb)} = \text{starch content - amylose content}$$

$$\text{Amylopectin content (\%bk)} = \frac{\text{amylopectin content (bb)}}{100 - \text{water content}} \times 100\%$$

Moisture content (AOAC, 1995)

The gravimetric method was used to take measurements. First, 1 gram of the sample was weighed in a weighing bottle (W1). It was then dried in an oven at 105 °C until a constant weight (W2) was reached. The weight reduction was recorded as the amount of water in the sample. The moisture content was calculated based on the dry weight of the material.

$$\text{Moisture content} = \frac{W1 - W2}{W1} \times 100\%$$

Physical Properties Analysis

Scanning Electron Microscopy (SEM) (Ratnaningsih et al., 2020)

Starch granule morphology was analyzed with scanning electron microscopy (SEM) (FEI Inspect TM S50, USA) using the method they described. A sample holder was used to place the starch sample. The sample holder was constructed from aluminum. It was a placeholder. The sample of starch was covered with gold-palladium. Gold and palladium were in a 60:40 ratio. The morphology of the starch granules was examined with a 10 kV acceleration potential. SEM analysis has the benefit of being able to determine the arrangement of starch granules.

Water Holding Capacity (WHC) testing (Subagio et al., 2003).

The weight of the empty centrifuge tube is measured as (a g), then each sample is weighed at 0.5 g as (b g) and poured into the centrifuge, with 3.5 g of distilled water added and vortexed. After vortexing, the sample is centrifuged at 2000 rpm for 5 minutes. After sedimentation, pour off or discard the water no longer mixed with the starch, then weigh the bottle along with the sample sediment (c g).

$$\text{WHC} = \frac{(c-a)-b}{b} \times 100\%$$

Oil Holding Capacity (OHC) (Subagio et al., 2003)

The dry centrifuge tube was weighed as (a g), then each treatment sample was weighed at 0.5 g as (b g) and poured into the centrifuge tube, adding 3.5 g of oil and vortex mixing. The purpose of vortexing is to ensure that the oil, starch, or RS3 could be properly combined. After vortexing, centrifuge at 2000 rpm for 5 minutes, pour off the oil that did not settle with the starch or RS3, and weigh the bottle along with the sample sediment as (c g).

$$\text{OHC} = \frac{(c-a)-b}{b} \times 100\%$$

Pasting properties (Yusra & Putri, 2023)

The pasting characteristics of RS3 gembolo were examined by researchers using the Rapid Visco Analyzer (RVA) method (Yusra & Putri, 2023). A total of 3.0 g (dry weight) of the sample was added to an RVA container, along with 25 g of distilled water. Using the RVA, measurements were obtained during the heating and cooling phases while maintaining a constant stirring speed of 160 rpm. The starch suspension was heated from 50 °C to 95 °C at 6 °C per minute, then maintained at 95 °C for 5 minutes. Once the heating phase is complete, the starch paste enters the cooling phase, during which the temperature is reduced from 95 °C to 50 °C at 6 °C per minute and held at that temperature for 2 minutes. The gelatinization profile curve is plotted by the RVA instrument. On the y-axis of this curve are viscosity values (cP). Additionally, it pertains to the variation in temperature (°C) during heating and cooling phases. The x-axis shows these temperature changes. During heating and stirring, researchers use the RVA to assess starch viscosity. RVA serves a useful purpose by characterizing starch properties.

Starch color (Yusra & Putri, 2023)

Color testing was conducted using Hunter color notation. Color testing was performed objectively using a Minolta chromameter (Conika Minolta CR-400). The sample was placed on the sample holder. The measuring lens was then placed over the sample, and measurements were taken of the L, a, and b values, which were subsequently used to calculate A (delta) E.

The difference between L*, a*, and b* of the sample object and the standard object is calculated using a single value, called Delta E*. Color testing produces parameter

values: L*, a*, and b*. Researchers use L* (brightness), a* (redness or greenness), and b* (yellowness or blueness) to measure Hunter tristimulus values.

Yield (AOAC, 1995)

The yield formula is calculated using the following formula:

$$\text{Yield} = \frac{\text{mass of gembolo starch (g)}}{\text{mass of sample placed in the drying device (g)}} \times 100\ \%$$

Experimental Design

This study employed a completely randomized design (CRD), with autoclaving-cooling cycles conducted 1, 2, 3, 4, and 5 times. Five samples were tested, each with three repetitions. The controlled environment in which this study was conducted ensured that any observed differences in effects were attributable solely to the treatments and that the effects were assumed to be homogeneous. The observed variables comprised starch color, granule morphology (SEM), yield, WHC, OHC, pasting properties, moisture content, starch content, amylose content, amylopectin content, and RS content. Every treatment was replicated thrice.

Data Analysis

The test results will be analyzed using the Analysis of Variance (ANOVA) test at a significance level of 95% (SPSS for Windows, version 25.0). If an effect is identified, a follow-up evaluation will use the Duncan Multiple Range Test at a significance level of 5% (α = 0.05) to determine significant differences between treatments. Groups that are statistically different will be marked with distinct letters (a, b, c).

RESULTS AND DISCUSSIONS

Isolation of Natural Gembolo Starch

The natural starch sample used in this study was obtained from gembolo tubers originally from Malang, East Java. Gembolo starch was extracted using the method described by Kusnandar *et al*. (2021). Table 1 presents the physical and chemical properties of the natural gembolo starch produced.

Gembolo starch yields 13.08% after extraction, while sweet potato starch yields 15.8%. Starch yield is calculated as the ratio of the weight of the obtained starch to the weight of the cleaned gembolo. The starch produced has a clean white appearance. Gembolo starch will undergo autoclaving-cooling treatment, and physical modification of gembolo starch through autoclaving-cooling treatment includes gelatinization and retrogradation. This study uses 1, 2, 3, 4, and 5 cycles of autoclaving-cooling treatment.

Chemical Characteristics

Resistant Starch (RS) Content (db)

RS is a type of starch that cannot be broken down by digestive enzymes. It is resistant to stomach acid, allowing it to reach the large intestine, where probiotic bacteria ferment it (Setiarto *et al*., 2019). The RS and starch contents of gembolo are presented in Table 2.

Modifying gembolo starch with 1 to 5 treatment cycles significantly increased the value from 40.71±1.21 to 74.00±1.03. This was due to the formation of RS3 in the modified starch. The RS of natural gayong starch was 27.79%, and that of natural Goroho banana starch was 39.35%.

The recrystallization of amylose molecules increases RS during autoclaving-cooling during retrogradation; each heating can increase the degree of starch gelatinization, and repeated cooling triggers more retrogradation. During the re-association of amylose-amylose, amylose-amylopectin, and amylopectin-amylopectin, the cooled paste forms a hard gel. Retrogradation alters the starch structure, leading to the formation of new crystalline structures resistant to enzymes.

Autoclaving-cooling treatment of starch can improve the arrangement of crystal granule fractions, thereby increasing granule stability, including starch resistance to amylase enzyme activity. This increased granule stability enables RS formation.

The results of this study align with previous research on Garut starch, where autoclaving-cooling cycling treatment can increase the formation of RS 3 with a gelatinization time of 15 minutes and modifications of 3 cycles at 10.91% bk and 5 cycles at 12.15% bk (Sugiyono, Faridah Nur, and Pratiwi 2009), tunggal bean starch experienced an increase in RS from 32.14% to 41.26% using autoclaving-cooling cycling for 1 cycle over 15 minutes (Ratnaningsih *et al*., 2020), natural tacca starch had an RS of 4.25±0.2%, then autoclaving -cooling with several cycles: 1 cycle RS 41±9.6%, 2 cycles 53±14.13, 3 cycles 42±23.67%, 4 cycles 39±7.2, and 5 cycles 40±6.37% (Herawati *et al*., 2020)

Starch content (%)

Starch, a complex carbohydrate, is composed of multiple units of D-glucose. D-glucose is a type of homoglucan or

Table 1. Physical and chemical properties of natural

Chemical properties	Content RS (% db)	40.71 ± 4.68
	Starch content (% db)	78.54 ± 1.37
	Amylose content (% db)	44.05 ± 0.61
	Amylopectin content (% db)	34.49 ± 1.13
	Moisture content (% db)	13.10 ± 0.40
Physical properties	Yield (%)	13.08
	WHC (%)	77.43 ± 7.54
	Color	
	L*	90.13 ± 2.74
	a*	-5.98 ± 0.29
	b*	9.39 ± 3.66
	Pasting properties/ Viscosity (cP)	10018

Note: L*= color brightness level, a*= positive is red and a negative is green. b*= positive is yellow and b*= negative is blue.

Table 2. RS Content and starch content

Frequency	Content RS (% db)	Starch content (% db)
Natural starch	40.71 ± 4.68^{a}	78.54 ± 1.37^{a}
Cycle 1 (A1)	50.89 ± 6.06^{b}	73.66 ± 0.18^{a}
Cycle 2 (A2)	54.18 ± 4.53^{b}	69.97 ± 8.05^{a}
Cycle 3 (A3)	65.65 ± 1.98^{c}	69.57 ± 6.40^{a}
Cycle 4 (A4)	67.99 ± 3.74^{cd}	69.27 ± 3.70^{a}
Cycle 5 (A5)	74.00 ± 1.03^{d}	68.89 ± 6.48^{a}

Note: Different letters in the same column indicate significant differences ($P < 0.05$).

glucopyranose that consists of two primary biomacromolecules, specifically amylose and amylopectin. The amount of starch typically decreases after autoclaving and subsequent cooling. The reason for this phenomenon is that continuous exposure to elevated temperatures and subsequent cooling cycles degrade glycosidic linkages within starch molecules. These chemical bonds are present within the molecules of amylose and amylopectin. As a consequence of this process, a portion of the starch is broken down into simple sugars, reducing the overall starch concentration. Moreover, subjecting starch to temperatures above its gelatinization point can damage the crystalline arrangement of starch molecules, leading to the liberation of amylose from within the starch granules, as indicated by Nur Faridah and S. L. Jenie (2016). In Table 2, data are presented on the amount of resistant starch (RS) in gembolo and its overall starch content.

Amylose and Amylopectin Content

Starchy foods are categorized into four groups based on the amount of amylose they contain: extremely low (less than 10%), relatively low (10%-20%), moderate (20%-24%), and high (exceeding 25%). Table 3 displays the levels of amylose and amylopectin found in the samples.

Amylose consists of a straight structure made up of α-D-glucose units linked together by α-1,4-glycosidic bonds. As can be observed in the table provided above, it is evident that the inclusion of autoclaving-cooling cycles did not increase the amylose content; instead, it decreased from an initial value of 40.90% to 35.49%. According to the findings presented by Shah and colleagues in 2016, the process of double autoclaving-cooling not to have any impact on the amylose levels found in rice starch. Similarly, research by Herawati and her team in 2020 indicated that the amylose content of takka starch

Table 3. Amylose and amylopectin

Frequency	Amylose content (% db)	Amylopectin content (% db)
Natural starch	44.05 ± 0.61[b]	34.49 ± 1.13[a]
Cycle 1 (A1)	40.91 ± 0.79[ab]	32.75 ± 0.98[a]
Cycle 2 (A2)	39.54 ± 0.52[ab]	34.89 ± 1.29[a]
Cycle 3 (A3)	38.73 ± 0.37[ab]	34.45 ± 0.58[a]
Cycle 4 (A4)	36.73 ± 5.58[a]	33.47 ± 3.05[a]
Cycle 5 (A5)	35.49 ± 5.63[a]	32.16 ± 0.89[a]

Note: Different letters in the same column indicate significant differences (P<0.05)

decreases during the autoclaving-cooling process, while the amylose content remains largely unaffected.

Table 3 shows that autoclaving-cooling cycles reduce the amylose and amylopectin content. This reduction happens after several cycles. The reduction is due to two main mechanisms: first, the hydrolysis of glycosidic bonds, and second, retrogradation and the formation of resistant starch. Glycosidic bond hydrolysis occurs when autoclaving breaks the glycosidic bonds in amylose and amylopectin through thermal hydrolysis, especially during repeated cycles. This breakdown converts starch into dextrins or simple sugars (such as glucose and maltose), thereby reducing the levels of both components. (Kusnandar, Feri *et al*., 2015). Retrogradation and resistant starch formation occur when repeated autoclaving-cooling cycles trigger retrogradation, in which amylose molecules and amylopectin chains rearrange to form a compact crystalline structure (type III resistant starch). This structure is not detected by conventional chemical analysis, so both levels appear to decrease even though the total starch mass remains constant (Nur Faridah & S. L. Jenie, 2016).

Moisture Content

The moisture content after modification did not differ significantly around 8% because, during autoclaving-cooling treatment, water in the material undergoes only phase transitions (gelatinization and starch retrogradation); total water content does not increase or decrease significantly because the system is closed and no water is added or removed from the outside. Table 4 shows the moisture content.

The autoclaving-cooling process is carried out in a closed system, so that water in the material and the autoclave environment does not freely enter or exit. After cooling, water remains in the system. Therefore, the total water content remains unchanged despite internal structural changes (resistant starch formation).

Physical Characteristics

Scanning Electron Microscopy (SEM)

The outcomes obtained from the examination using a scanning electron microscope for both unaltered and chemically altered starch are depicted in Figure 1. Illustration: Findings from the analysis conducted using scanning electron microscopy. The natural form of starch granules is characterized by the elongated, regular shape of natural gembolo starch, with all starch particles displaying a smooth appearance, as detailed in the study by Kusnandar, Mutmainah, and Muhandri in 2021. After the process of autoclaving and subsequent cooling, it can be observed that the modified starch granules exhibit an increase in size and an irregular shape. This alteration occurs during heating and cooling, leading to the degradation of amylose and amylopectin molecules. Subsequently, the amylose chains retrograde as a result of

Table 4. Water content

Frequency	Moisture Content (%)
Natural starch	13.10 ± 0.40[b]
Cycle 1 (A1)	8.37 ± 0.22[a]
Cycle 2 (A2)	8.10 ± 0.24[a]
Cycle 3 (A3)	8.91 ± 0.05[a]
Cycle 4 (A4)	8.69 ± 0.77[a]
Cycle 5 (A5)	8.61 ± 0.53[a]

Note: Different letters in the same column indicate significant differences (P<0.05).

Table 5. Pasting Properties

Frequency	Peak (cP)	Trough (cP)	Breakdown (cP)	Final Viscosity (cP)	Setback (cP)	Peak Time (cP)	Pasting time (°C)
Natural starch	10018	5342	4676	7466	2124	6.8	67.1
Cycle 1 (A1)	4657	3404	1253	4674	1270	7.87	69
Cycle 2 (A2)	2443	2338	105	3593	1255	9.2	68.8
Cycle 3 (A3)	2330	2331	1300	3630	1299	12.87	69.45
Cycle 4 (A4)	5197	3800	1397	5115	1315	7.33	67.45
Cycle 5 (A5)	2265	2165	100	3174	1009	9.07	69.35

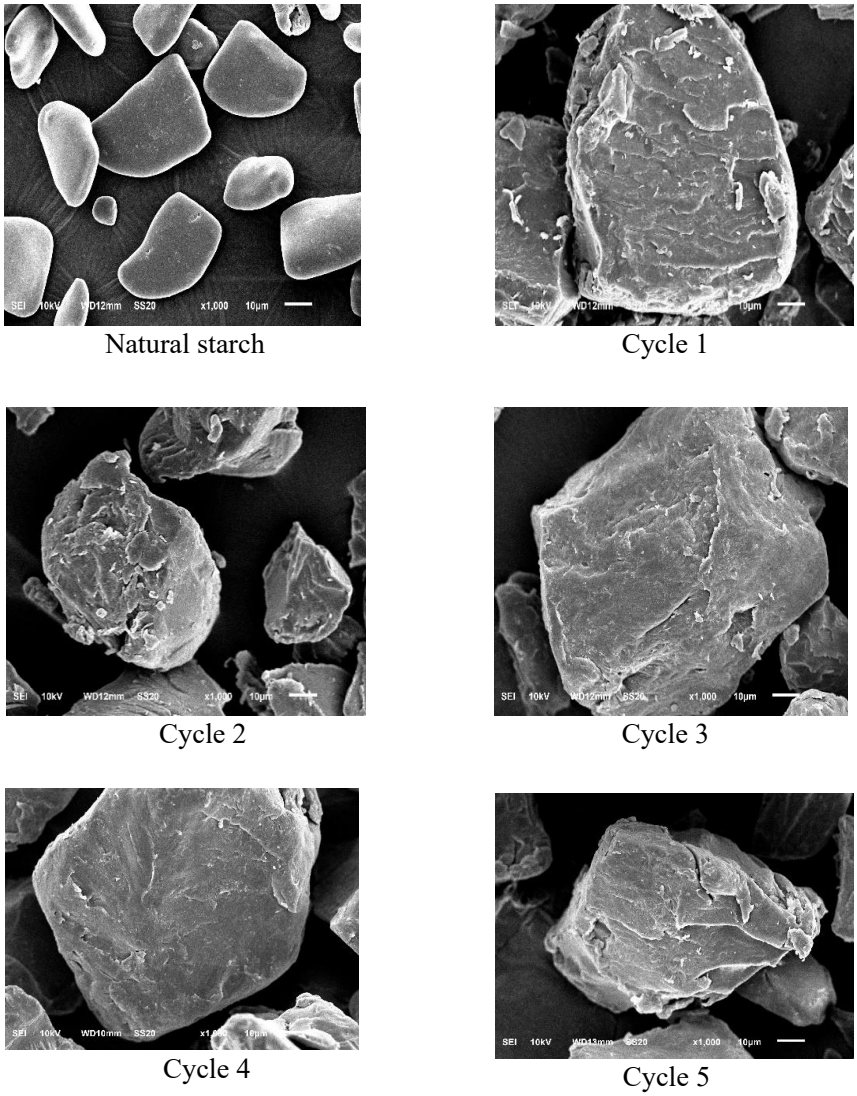


Figure 1. Scanning electron microscopy of gembolo starch and autoclaving-cooling modified starch at 1000× magnification.

Table 6. Water holding capacity and oil holding capacity

Frequency	WHC (%)	OHC (%)
Natural starch	77.43 ± 7.54[a]	78.14 ± 8.67[a]
Cycle 1 (A1)	195.49 ± 4.49[b]	78.57 ± 1.62 [a]
Cycle 2 (A2)	195.16 ± 4.49[b]	80.65 ± 3.35 [a]
Cycle 3 (A3)	202.85 ± 2.48[b]	80.27 ± 9.06 [a].
Cycle 4 (A4)	221.00 ± 7.94[c]	80.30 ± 1.13 [a]
Cycle 5 (A5)	221.65 ± 8.32[c]	81.05 ± 7.36 [a]

Note: Different letters in the same column indicate significant differences ($P < 0.05$).

starch granule rupture. During the process of gelatinization, the starch granules undergo an irreversible breakdown. The structural alterations resulting from the retrogradation of amylose chains lead to a reorganization of the starch into a more intricate helical form.

Pasting Properties

Natural starch exhibits maximum viscosity, breakdown, and setback. Natural starch paste has a high peak viscosity (10018 cP) with a very high breakdown viscosity value, indicating that natural starch is unstable when heated. Natural starch also exhibits a fairly high tendency for retrogradation, as indicated by its setback value (2124 cP).

Viscosity is the ability of a liquid to resist flow; the highest viscosity value of natural starch indicates that natural starch has a tendency to thicken easily. One of the factors that significantly influences the high viscosity is the temperature during the gelatinization process, which strengthens the bonds formed, thereby increasing viscosity (Yusra & Putri, 2023). Generally, the viscosity profile of modified starch has lower viscosity values during the heating and cooling phases than natural starch. The reduction in viscosity is probably due to damage to starch granules and partial solubility caused by the high temperatures of the autoclave process. The following is Table 5 of pasting properties

Water-Holding Capacity (WHC)

Water Holding Capacity (WHC) is the ability of starch to absorb water at room temperature. WHC values for natural and modified starch are shown in Table 6. WHC and OHC.

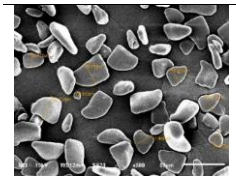
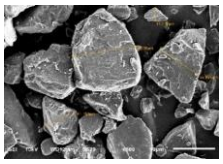
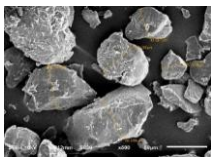
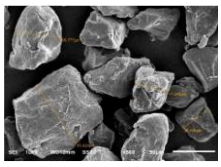
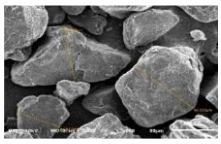
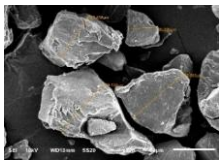
Using autoclaving-cooling with multiple cycles can increase WHC due to changes in starch structure during autoclaving, which heats the material at high temperatures under pressure, causing starch gelatinization. Subsequently, during cooling, retrogradation occurs, the reformation of a more stable, ordered structure. Repeated autoclaving-cooling cycles strengthen the structure,

Table 7. Colors

Frequency	Color		
	L^*	a^*	b^*
Natural starch	90.13 ± 2.74[c]	-5.98 ± 0.29[a]	9.39 ± 3.66[b]
Cycle 1 (A1)	80.44 ± 1.48[ab]	0.23 ± 0.27[b]	2.97 ± 1.13[a]
Cycle 2 (A2)	81.62 ± 1.70[b]	0.23 ± 0.11[b]	1.72 ± 0.46[a]
Cycle 3 (A3)	78.49 ± 0.32[ab]	1.18 ± 0.50[c]	3.91 ± 0.19[a]
Cycle 4 (A4)	77.83 ± 2.71[a]	0.78 ± 0.35[c]	3.52 ± 0.52[a]
Cycle 5 (A5)	78.65 ± 1.58[ab]	1.17 ± 0.20[c]	3.48 ± 0.32[a]

Note: L*= color brightness level, a*= positive is red and a= negative is green. b*= positive is yellow, and b*= negative is blue.

Table 8. The averae of granule size and the image of the granule of gembolo starch and autoclaving modified starch taken with electron microscopy at 500× magnification.

Frequency	Granule size (μm)	Average (μm)
 Natural starch	8.35 - 26.75	17.55
 Cycle 1	17.21 - 75.23	46.22
 Cycle 2	30.17 - 58.20	44.18
 Cycle 3	54.67 - 93.02	73.84
 Cycle 4	54.67 - 93.02	73.84
 Cycle 5	35.22 - 79.13	57.17

making it more stable and capable of holding more water. The resistant starch content increases with autoclaving-cooling cycles, which are associated with increased starch crystallinity. This resistant starch has a higher water-binding capacity, thereby increasing WHC (Puspawati *et al*., 2024).

Based on Table 6, natural starch has a lower WHC than modified starch. WHC increased 221% at cycle 5 compared to the control. This is because starch gelatinizes during autoclave heating. Using autoclaving-cooling results in larger granule sizes, thereby increasing water absorption.

Oil-Holding Capacity (OHC)

The oil-holding capacity (OHC) is the ability of a substance to absorb and retain oil within its structure. After multiple cycles, the OHC remains relatively stable and shows an increase due to various factors. First, changes occur in the structure of starch granules, for example, through modification processes such as heating. These processes cause harm to the granules by either damaging them or stretching them out. This damage to the starch structure results in a greater degree of openness or porosity, facilitating the penetration of oil and its enhanced binding within the granules. In a second step,

the hydrogen bonds that exist between the molecules of amylose and amylopectin are either weakened or disrupted. Consequently, the configuration of the starch molecules loosens up, leading to a decrease in density and an increase in the ability to soak up oil. In addition, the process of heating can lead to the expulsion of amylose and amylopectin molecules from the granules, thereby enhancing the granules' capacity to absorb oil, as demonstrated in the study by Shahira and colleagues (2023). The OHC remains at a consistent level as it progresses through the entire cycle. The subsequent information pertains to Table 6, which displays the Oil Holding Capacity (OHC).

Color

Color values (L*, a*, and b*) are presented in Table 7. Starch color differs significantly, with L* values ranging from 77.83 to 90.13, with natural starch having the maximum brightness. The maximum a* value is 1.18 and b* 9.39; the starch color is predominantly white because it comes from gembolo that has undergone repeated washing processes, resulting in a white color.

CONCLUSION

The conclusions of this study are as follows: Modification of gembolo starch through autoclaving-cooling cycles can alter the physical properties of starch, including increasing the RS content, reducing starch content, decreasing amylose and amylopectin content, no significant change in moisture content, altering the shape and size of starch granules, reducing viscosity, increasing WHC, and decreasing OHC. Gembolo starch using 1 to 5 autoclaving-cooling cycles can increase the RS content from 40.71% to 74.00%, experiencing a significant increase, which is the highest RS content. Gembolo can be used as a source of RS3.

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