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Preparation and Characterization of Type 3 Resistant Starch from Cilacap Breadfruit (*Artocarpus altilis* (Parkinson) Fosberg) Starch

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***Abstract*— The Cilacap breadfruit grown in Indonesia, possesses superior properties, although the application as a carbohydrate source is not very popular compared to other resources, including banana, corn, and cassava. Furthermore, they contain relatively high fiber content, hence the possibility of further development into beneficial resistant starch. This study aims to investigate the Cilacap breadfruit starch, and the type 3 resistant starch (RS3) produced at high temperatures, using a 3 cycle *Autoclaving-Cooling* method with strong acid hydrolysis. The autoclave was set at 100°C, 121°C, and 140°C, respectively, while the storage temperature was 4°C for 24 h and 48 h, followed by hydrolysis with 0.1 M HCl at 40°C for 24 h, and then neutralization using 1 M NaOH, at 4°C for 24 h. Therefore, both samples were evaluated based on chemical, functional, and physical characteristics. The results showed chemical changes in the Cilacap breadfruit starch converted to the resistant starch under similar autoclave treatment (100, 121, and 140°C). However, the extended cold storage (48 h) prompted a significant decline ($p \leq 0.05$) in carbohydrate, and starch levels, alongside increased antioxidant activity, insoluble fiber, and total dietary fiber content, compared to samples stored for 24-h. Furthermore, autoclaving at 140°C and cooling at 4°C for 48 h yielded products with the highest antioxidant activity (9.11%). Also, higher temperatures (121°C and 140°C), and subsequent storage at 4°C for 24, and 48 h causes an increase in the WAI and OHC values of native starch and modified the structure of the granule. The native starch has a type B crystal pattern.**

***Keywords*—Cilacap breadfruit starch; RS3; autoclaving-cooling; acid hydrolysis; chemical; functional and physical properties.**

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I. INTRODUCTION

Breadfruit is a tropical plant grown under numerous land conditions or agroforestry systems [1] and also adapts properly to tropical regions with temperatures of 21-23°C, and rainfall of 1525-2540 mm [2]. This is known to have originated in Polynesia and spread to various tropical regions worldwide, including the Caribbean, Africa, America, the Pacific, and several other countries, including Indonesia and India [3], [4], characterized by good biodiversity [5]. This is beneficial as Indonesia has diverse local food sources [6]. Indonesia is a tropical country that has great potential in developing agricultural commodities-based food [7]. The advantage of breadfruit trees includes the ability to grow well in dry and marginal areas [8], and also the capacity to bear fruits all year, with a huge harvest between February and June, and supplementary yield from July to September [5], [9]. Furthermore, one tree unit can produce about 100 to 600 fruits, at an average of 200 to 300 [2], [10] depending on soil and climatic conditions [10], as well as the growth area, age, variety, and plant properties [3]. Breadfruit is high in carbohydrates and is also a source of vitamins and minerals [2], [4], low in fat [3], and rich in fiber. The majority of carbohydrate food sources are in the class of cereals and tubers [11], but others are belonging to the fruit category, including pumpkins [12], breadfruit [11], and bananas

[13],[14]. However, breadfruit has not been utilized extensively.

This resource is converted into flour and starch form to extend shelf life [2], [15], although the latter has wider application in food and pharmaceutical industries [2],[11]. Starch can be isolated from various carbohydrate sources, one of which is breadfruit (*Artocarpus altilis* (Parkinson) Fosberg) [16]. The high carbohydrate content in breadfruit instigates the potential as an alternative resource for starch isolation [15]. Numerous studies have been conducted to evaluate the chemical properties, while research on the food fiber content, production of resistant starch, and antioxidant activity have not been performed, especially on Cilacap breadfruit from Indonesia.

This plant is grown in the Cilacap district of central Java and is one of the superior species in the region. Furthermore, analysis performed by the Bogor Botanical Gardens Conservation Center, Indonesia, characterized the *Artocarpus altilis* (Parkinson ex. F.A. Zorn) Fosberg species, from the Moraceae tribe as bare (smooth surface without trichomes). The advantages of Cilacap breadfruit include the fast-growing tendency, ability to produce large fruits, and grow under a wide variety of ecological conditions [17]. Moreover, the applications are currently limited to consumption, after peeling, and subsequently fried, boiled, or used to create crepes and various foods. Furthermore, advanced processing and starch use are not common, although there have been reported health benefits in the fruit form, as seen with pumpkin [18]. This is due to the presence of several compounds, including alkaloids and saponins, characterized by the capacity to reduce blood glucose in people with diabetes mellitus and uric acid levels [10]. Therefore, it is necessary to increase the utilization of breadfruit starch in new products to impact the health benefits.

The manufacturing process possibly yields resistant starch (RS), unaffected by gastrointestinal digestion and amylolytic hydrolytic enzymes, after 120 minutes incubation period in the small intestine. Subsequently, fermentation ensues in the large intestine [19], [20], [21] due to the activity of microorganisms or probiotic bacteria [22]. Furthermore, RS acts as food for beneficial gut bacteria after transiting to the colon and is thus referred to as prebiotics [23], [24]. The beneficial physiological effects include hypoglycemic properties, anti-hypercholesterolemic effects, and inhibition of colon cancer, fat accumulation, and gallstone formation. Also, there have been reports on the capacity to increase mineral absorption [25], [26], and contribute as a prebiotic [22], [23], [24], [26]. Furthermore, consumption is shown to cause a decline in the glycemic index (GI) of diet, thus enhancing the beneficial effects for individuals with type 2 diabetes, glucose intolerance, and obesity [19],[27].

There are five types of RS (RS1, RS2, RS3, RS4, and RS5) [22], [28], [29], categorized based on the preparation method and botanical sources [24]. Furthermore, RS3 is one of the most common varieties used in the food industry and is also widely available commercially. The raw material has been extensively researched to evaluate the overall physiological characteristics of resistant starch [24]. Specifically, RS3 (starch retrogradation) is produced using the gelatinization process, through the exposure of raw materials to crystal nucleation for at least one cycle, followed by the propagation process [30], and storage at cold temperatures [31]. The most widely used method involves combining high-pressure heating (autoclaving) with cooling and hydrolysis with enzymes or acid, but hydrolysis with acid is estimated to be cheaper and easier [22]. The production process is conducted out on a variety of raw materials, including starch sourced from high amylose corn [19], [32], sago [24], [26], [33], arrowroot [34], [35], cassava [36], rice [37], banana [13], [14], ginger [38], red bean [39], and cowpea [40]. However, RS3 has not been processed from Indonesian Cilacap breadfruit. The objective of this study was to investigate the chemical, functional, and physical characteristics of RS3 produced from the native starch of Cilacap breadfruit, Indonesia, which was treated at various autoclaving temperatures (100, 121, 140°C), followed by cold storage at 4°C for 24 and 48 hours to ensure retrogradation, and acid hydrolysis.

II. Materials And Methods

A. Materials

The main ingredient in this study was Cilacap breadfruit starch extracted from the ripe fruit of Cilacap tree. This sample was harvested in Jetis hamlet, from an individual's garden in Klepu village, Ceper sub-district, Klaten Regency, Central Java, Indonesia. Therefore, the starch was extracted through isolation by sediment separation, using a wet-milling method [11], with natural modification (devoid of bleaching chemicals) [17]. The starch utilized was the white bottom sediment, and the pre-analysis chemicals used were purchased at the Chemix chemical shop, Bantul Yogyakarta, Indonesia.

B. Production of Resistant Starch from Cilacap Breadfruit

1) Autoclaving-Cooling Cycles: The gelatinization-retrogradation process required the autoclaving-cooling of Cilacap breadfruit starch, prepared using the autoclaving-cooling method [19] with modification [16]. This involved the dissolution of a 150 g starch sample in 300 ml aquadest. Meanwhile, another 300 ml of distilled water was heated to a boil and slowly poured into a starch solution while constantly stirring (total starch: water, 1:4, w/v). Furthermore, the mixture was autoclaved at different temperatures (100, 121, 140°C) for 30 minutes and allowed to stand for 1 hour at room temperature. The resulting paste was then stored at 4°C, over 24 and 48 h storage period. The autoclave and subsequent cooling process were repeated 3 times, and the solution was then dried at 45°C in a cabinet dryer, before milling, and sieving (100 mesh).

2) Acid Hydrolysis of Retrograded Starch Cilacap Breadfruit: Acid hydrolysis of retrograded starch was performed [19]. This process was performed in 3 cycles, where samples were dissolved in 0.1 M HCl solution (starch: HCl, 1:4) (w/v) for 24 h, in a water bath shaker set at 40°C. Furthermore, the acid-grade retrogradation starch porridge was neutralized with 1 M NaOH solution. This product was then stored at 4°C for 24 h before drying in a dryer cabinet set at 45°C, ground, and sieved (100 mesh).

3) The Sequence of The Research Process for Breadfruit RS3 Production: Breadfruit resistant starch was produced according to the autoclaving-cooling method [19] with modifications [16]. The autoclaves used were of different types, set at three varied temperatures. First, a conventional autoclave equipped with temperature and pressure gauge and a gas stove heater was used to achieve 100°C. Second, 121°C was generated using the Eastern Medical type of EA-632, with an electric heat source, while the third at 140°C was acquired with an electric heat source created by Prof. Wahyudi, from the Separation Laboratory, Faculty of Chemical Engineering, Gadjah Mada University, Yogyakarta. Therefore, the RS3 starch production procedure is described as follows:

- *Preparation of Resistant Starch:* A total of 150 g breadfruit starch was weighed using a digital scale into a glass beaker before dissolving with 300 ml distilled water. Meanwhile, another 300 ml of aquadest was heated and poured slowly into the slurry while stirring to attain homogeneity, at starch: water of 1:4 (w/v). The solution is then tightly enclosed with aluminum foil before covering with a plastic measuring 0.06 mm thick, and the lid is fastened with a rubber band. Furthermore, the setup is heated in an autoclave at 100°C for 30 minutes to ensure gelatinization, followed by cooling at room temperature in a beaker glass for ± 1 hour.
- *Cold Storage and Autoclaving-Cooling three Cycles:* The gelatinized starch is cooled in a refrigerator set at a temperature of 4°C for 24 and 48 hours, respectively, to achieve retrogradation. Subsequently, the samples are autoclaved again, and the autoclaving-cooling process is performed in 3 cycles.
- *Drying, Grinding and Sieving of Starch Retrograded:* The samples are further transferred to stainless steel pans, measuring 30x30x3 cm in size, and dried at 45°C in a cabinet dryer. Therefore, the product is ground and sieved with a 100 mesh.
- *Hydrolysis and Incubation in 0.1 M HCl Solution:* The starch retrograded powder is introduced to a glass beaker and a solution of 0.1 M HCl (starch: HCl = 1: 4 w / v) was added before incubating at 40°C, using a Julabo SW22 water bath shaker for 24 hours.
- *Neutralization, Drying, Grinding, and Sieving of Resistant Starch:* The starch in acidic solution was neutralized using 1 M NaOH solution and measured with a digital pH meter HI 2210, and then stored at 4°C for 24 hours. Successively, the sample was dried at 45°C in a drying cabinet before grinding and sieving with a size 100 mesh.

The RS3 production process conducted at 121°C and 140°C autoclaves were generally similar to the 100°C treatment, although 140°C differed slightly. This was due to the distinct autoclave size, shape, and volume, being the smallest, measuring 16.5 cm in height, at a diameter of 24.5 cm, with the hole estimated at 13.8 cm. Therefore, the production process was conducted only once for 30 minutes, using a 500 ml glass beaker (10 cm in diameter and 11.8 cm high), comprising 100 g of starch samples and 400 ml aquadest. Hence, 300 g is treated over 3 exposures, leading to more time, physical exertion, and energy consumed. The lid adjacent to an iron bar (diameter 1.1 cm, length 8 cm) integrates with the lid and assists in heat penetration. This component automatically enters the beaker glass and tears the protective aluminum foil. Therefore, cooking at 140°C and very high pressure (± 2.2 bar) instigates the rise and exit of starch solution through the iron bar, further causing a decline in the yield of resistant starch. In contrast, the other autoclaves (set at 100 and 120 °C) process samples on a single treatment procedure, using two 1000 ml glass beakers (@ 150 g of sample with 600 ml aquadest).

C. Analysis

The chemical analysis involves measuring the water content through the drying method [41] and evaluating ash composition with the dry aching approach [41]. The fat constituent was assessed using the Soxhlet method [41], and micro Kjeldahl was applied in the determination of protein content [41], while carbohydrate was analyzed by difference, starch content [42]. In addition, the antioxidant activity was measured using the DPPH method [43], while food fiber (soluble, insoluble, and total fiber) analysis [44]. The evaluation of functional properties: water absorption index (WAI) [19] and oil holding capacity (OHC) [45], as well as physical characteristics, including the Cilacap breadfruit crystal starch types involved an X-ray diffractometer (Shimadzu XRD-6000, Shimadzu Corp. Japan) [11]. Furthermore, Scanning Electron Microscopy (Hitachi, SU-3500) was used to assess the granule morphology [40].

D. Statistical Analysis

This study used a completely randomized design, with treatments including the autoclaving temperature (100, 121, and 140°C) combined with storage (24 and 48 h) at 4°C, followed by acid hydrolysis. The respective effects were determined by analysis of variance (ANOVA) using a statistical analysis system (SAS) version 9.4, and 5% ($p \leq 0.05$) levels were considered significant. Subsequently, a Duncan test was performed.

III. Results and Discussion

A. Chemical Characteristics

Table 1 shows the proximate analysis conducted on the native starch as well as the type 3 resistant starch. The breadfruit starch produced for this study was obtained from the white bottom sediment through isolation by natural separation (without any chemicals). This procedure yields two forms of starch, including the top (L*87.59, brownish color) and bottom precipitate (L*94.11, white color) varieties [17].

The native Cilacap breadfruit starch possessed a moisture content of 6.25%, which is lower than the value reported in previous studies, at 13.05% [15]; 10.83% [46]; 10.91% [11]; 13.24% [47] and 6.6% [48]. However, low water content indicates the potential for high shelf life in dry products, e.g., flour [46], resulting from the ability to restrain microbial growth [48],[49], and subsequently prevent damage to food.

The result from the native starch showed an ash content of 0.44%, which is higher than the report [15] at 0.35%, but lower from some researchers [8], [11], [46] at 1.77, 0.99, and 0.49%, respectively. Moreover, the fat content was 0.08%, lower than the report [15] at 0.51%, [46] at 0.39%) and [47] at 0.46%, but higher than the report [11] at of 0.04%. This result reflects the low-fat status [3], [11], [50]. In addition, the protein content was slightly higher (1.84%) in comparison with the outcome of other researches, including 1.61% [15], 0.53% [46]. 1.39% [11] and 0.31% [47]. The carbohydrate content is relatively high (91.40%), compared to the value recorded in breadfruit species obtained from other countries, including 84.48% [15], 86.48% [46], and 56.68% [11]. This verifies the tendency for use as an alternative carbohydrate source [2], [3], [4], [10], [47], [50]. Furthermore, the variations in chemical properties are possibly initiated by the differences in soil conditions, climate, cultivar varieties, fruit maturity level [47], and harvest age [34], as well as disparities in the extraction and drying processes [2]. The starch content of native Cilacap breadfruit was 82.85%.

TABLE I
CHEMICAL PROPERTIES OF CILACAP BREADFRUIT RESISTANT STARCH TYPE 3

Sample	Components						
	Water (%)	Ash (%)	Fat (%)	Protein (%)	Carbohydrate (%)	Starch (%)	Antioxidant activity (%)
Native starch	6.25^{c}±0.01	0.44^{f}±0.01	0.08^{d}±0.02	1.84^{ba}±0.02	91.40^{a}±0.03	82.85^{a}±0.16	3.59^{d}±0.59
100°C/24 h	4.74^{e}±0.04	2.83^{c}±0.04	0.07^{d}±0.01	1.92^{a}±0.05	91.05^{a}±1.09	79.47^{b}±0.04	7.09^{c}±0.01
100°C/48 h	5.12^{d}±0.08	2.67^{e}±0.01	0.06^{d}±0.01	1.95^{d}±0.02	90.19^{b}±0.09	80.44^{b}±0.82	7.08^{c}±0.02
121°C/24 h	7.38^{b}±0.08	3.19^{a}±0.02	0.24^{b}±0.05	1.62^{bac}±0.14	87.57^{d}±0.19	77.82^{c}±0.15	8.01^{bc}±0.32
121°C/48 h	7.74^{a}±0.04	2.75^{d}±0.08	0.30^{a}±0.01	1.91^{a}±0.07	87.31^{d}±0.17	66.99^{d}±0.73	8.11^{bac}±0.80
140°C/24 h	6.18^{c}±0.17	2.93^{b}±0.05	0.12^{c}±0.01	1.55^{bc}±0.44	89.21^{c}±0.61	82.36^{a}±1.03	8.41^{ba}±0.58
140°C/48 h	7.89^{a}±0.15	2.82^{c}±0.02	0.09^{dc}±0.2	1.31^{c}±0.04	87.88^{d}±0.15	81.80^{a}±0.94	9.11^{a}±0.83

This result is higher than the values reported [8] on breadfruit starch extract (57.80%) but lower than the level in sago [26] at 93.45%), and arrowroot [34].

The water content in RS3 Cilacap breadfruit products decreased to 4.74% and 5.12% after autoclave treatment at 100°C and cold storage at 4°C for 24 and 48 h, respectively. However, a relatively similar result was observed at 140°C, while the outcome at 121°C for 24 and 48 h, as well as 140°C for 48 h, increased. This is consistent with those previous reports [15] on chemically (acetylation and oxidation) and physically (heat moisture treatment) modified breadfruit starch. The result showed a decline in the starch water content, although there was a general increase in value. The levels in all samples of this current research were consistent with the standard requirement for various flour products, set by the Indonesian National Standard Agency. These include SNI number 3751 [51] and SNI number 3451 [52], stipulating a maximum of 14.5%.

The manufacture of RS3 caused an increase in the ash content across all treatments (from 0.44 to 2.67-3.19%). This result was due to an increase in minerals after the hydrolysis with HCl, followed by neutralization with NaOH [34]. Despite the insignificant protein and fat levels changes, this upsurge was evidenced by the ash content [11]. In addition, RS3 produced by autoclaving at various temperatures (100, 121, and 140°C) followed by cooling to 4°C possessed lower carbohydrate content than the native variety with longer cold storage, as observed in 48 h, compared to 24 h. This was due to the HCl hydrolysis treatment provided during the incubation process and the subsequent production of simpler hydrolysates, with a distinctively lower degree of polymerization [34]. A previous study [15] also reported reduced carbohydrate levels in modified breadfruit starch, while [14] observed a similar outcome with banana starch.

After RS3 production through the autoclaving-cooling-storing method, strong acid hydrolysis generally tends to reduce starch levels (from 82.85% in native to 66.99-82.36%).

The RS3 formation process at various temperatures (100, 121, and 140°C) allows for gelatinization of the original starch. This is characterized by heat damage to the starch granule structure, thus prompting the evacuation of amylose fraction from the granules after swelling irreversibly [19], [22], [32], [53]. Also, autoclaving causes granular degradation, thus reducing the starch levels [22], [54]. Furthermore, cooling at 4°C prompts a retrogradation (recrystallization) process, featuring the reassembly of starch-forming molecules by creating stable hydrogen bonds. This leads to the rigid structural appearance and the generation of rigid strong layers on the granule surface [35]. The amylose fractions tend to bond with each other again under recrystallization (retrogradation) conditions through the hydrogen bonds, thus recreating a strong double helix structure [22], [55].

The native Cilacap breadfruit starch showed antioxidant activity of 3.59%, indicating active antioxidant compounds [2], [3]. Also, a qualitative test of active compounds showed a positive outcome for alkaloids, flavonoids, steroids, saponins, tannins, and triterpenoids [56], alongside polyphenol compounds [8], [50], and phenolate enzymes [8]. The antioxidant activity is possibly measured using various techniques, including the DPPH method [57], as the most common type [58], especially for polyphenol evaluation [57]. This approach was selected due to the ease and speed of performance [58] compared to other assays. Moreover, antioxidants are substances or compounds that play a protective role in the body against oxidative damage or free radical attack. These include molecules containing one or more unpaired orbital electrons, assumed to induce reactivity [56], as seen in the capture of radical compounds. As reducing agents, they tend to inhibit the formation of singlet oxidants and bind to prooxidant metal ions [59]. The results show increased antioxidant activity in Cilacap breadfruit RS3. A similar outcome was also reported on fresh vegetables (eggplant, carrots, and broccoli), where the effect was enhanced after boiling for 3 minutes [59]. Also, [57] demonstrated an upsurge in total phenolic levels alongside the antioxidant activity of Melinjo fruit (*Gnetum gnemon*) extract. This outcome was intensified with the simultaneous rise in temperature from 45°C, 60°C and 75°C, where the highest yields was obtained at 75°C. A previous study [60] on the production of sugar derived from coconut *neera*. The result showed an increase in the total phenolic content and antioxidant activity with successively higher heating temperature (100, 105 110 and 115°C) and pH 8. This was possibly because the phenolic compounds present in the fresh materials are bound to cellular components, causing the damage of cell wall and other characteristic elements at high temperatures. Also, there is a simultaneous extraction of phenolic compounds, thus enhancing the antioxidant activity [57]. Similar outcome was reported in previous studies on RS3 rice starch production by autoclaving-cooling method, confirmed using DPPH assessment. In addition, polysaccharides in the form of starch possess one or more hydroxyl groups, and extra units are exposed during the autoclaving process. These components tend to be responsible for the antioxidant abilities, and higher temperatures lead to more hydroxyl exposure [43]. This outcome is similar to Cilacap breadfruit converted to RS3, where an increase in antioxidant activity increases the beneficial value as an ingredient in functional food, especially in therapy for people, with diabetes mellitus, due to the polyphenols present. This naturally formed component alongside phytoalexin are 3,5,4-trihydroxystilbene or better known as resveratrol with well-established antioxidant profile [61].

Fiber analysis (soluble, insoluble and total fiber) was performed on native and resistant starch (RS) Cilacap breadfruit, and the results are presented in Table 2.

The food or total fiber content was 2.73% in the native starch, consisting of 1.67% soluble and 1.06% insoluble variety. This outcome was higher than the previous reported [15] at 0.42%, and the crude fiber content of breadfruit flour varied, ranging from 0.55% [2] to 2.93% [62].

TABLE II
CHARACTERISTICS OF FOOD FIBER CILACAP BREADFRUIT RESISTANT STARCH TYPE 3

Sample	Components		
	Soluble Fiber (%)	Insoluble Fiber (%)	Total Fiber (%)
Native starch	1.67^{b}±0.04	1.06^{dc}±0.06	2.73^{e}±0.10
100°C/24 h	2.12^{a}±0.18	4.44^{d}±0.23	6.56^{bc}±0.38
100°C/48 h	1.79^{b}±0.13	4.41^{a}±0.07	6.20^{c}±0.20
121°C/24 h	2.27^{a}±0.08	4.70^{ba}±0.34	6.97^{ba}±0.42
121°C/48 h	2.30^{a}±0.14	4.93^{a}±0.03	7.23^{a}±0.17
140°C/24 h	1.06^{c}±0.03	4.06^{d}±0.04	5.12^{d}±0.03
140°C/48 h	1.11^{c}±0.02	4.15^{c}±0.04	5.26 ^{d}±0.05

The differences in food fiber levels have also been observed in arrowroot starch fiber *(Marantha arundinacea*), at 3.82%, encompassing 1.22% and 1.6% soluble and insoluble forms, respectively [35]. Other studies showed a total food fiber of 2.41%, comprising 0.95% soluble and 1.46% insoluble variety [34]. The variation in values recorded despite the similar carbohydrate source is possibly attributed to soil differences, growing locale and climate [46], fruit maturity level or age at harvest [2], [34], [46], and cultivars [46], [47], as well as the extraction process [2].

Pressurized cooking and subsequent cooling at 4°C, followed by acid hydrolysis for 24 hours and 48 hours had a significant effect on increasing the levels of food fiber (from 2.72 to 5.12-7.23%). The production process for RS3 Cilacap breadfruit substantially increased the total food fiber and RS levels. Also, high fiber and RS content caused a decline in starch digestibility [34], [63], and further positively impacted people with obesity and/or type 2 diabetes. This is due to the help provided in reducing food intake due to increased satiety and decreased blood glucose levels [54],[64]. Furthermore, low digestibility impacts the undigested content (food fiber and RS) by enzymes. These materials in the small intestine transit to the colon for fermentation by the colonic microflora to form beneficial compounds, comprising short-chain fatty acids (butyric acid, acetic acid, and propionic acid) [23], and other fatty acids, including valerate, isovalerate, and isobutyrate [65], hence, it reduces the pH in this area of the tract. The acidic atmosphere has a physiological impact on colon cancer prevention and positively contributes to overall gastrointestinal health. Furthermore, retrogradation starch produced by autoclaving-cooling (RS3) has been reported to possess ± 18% of high amylose in barley flour samples. This can reduce blood glucose levels in rats within the first 120 minutes [65],[66].

In this study, the increase in total food fiber was elevated by the possible contribution of insoluble fiber compared to the soluble variety. Table 2 shows an insoluble fiber content of 1.06% in native starch, which increased by 4.6 times (from 1.06 to 4.93%) after processing to RS3. Conversely, the soluble form was augmented by 1.4 times (from 1.67 to 2.30%).

B. *Functional Characteristics*

The functional properties were evaluated in the native and RS3 from Cilacap breadfruit, and the results are presented in Table 3.

1) Water Absorption Index (WAI): WAI increased significantly (0.52-3.22 g/g) at a higher autoclaving temperature of 100-140°C, followed by storage for 24 and 48 h. This phenomenon is possibly due to the high heat susceptibility of granules to damages. Also, there is elevated water retention capacity and swelling after cooling at room temperature, resulting in increased WAI. This value recorded for native starch was 0.52 g/g, followed by a continuous increase at autoclaving temperatures of 100°C and 121°C, over a storage period of 24 and 48 h (at 3.22; 3.47; 3.11and 2.83 g/g, respectively). However, a decline, although higher than the native, was observed at 140°C. This outcome results from the level of damage estimated to have reached the maximum under this treatment condition. Also, there is a tendency for melting during the gelatinization phenomenon [67].

2) Oil Holding Capacity (OHC): The oil holding capacity of native starch is 1.01 (g/g), and a lower value was reported after cooking at 100°C and 121°C and subsequent storage at 24 and 48 h. However, treatments at 140°C demonstrated an increase in value. The increase in OHC is attributed to the amylose-lipid complex formed due to oil trappings in the helix structure. This outcome also ensues from the greater exposure to hydrophobic parts of starches or the presence of capillary force on the amorphous part, at higher autoclaving temperatures. However, the capillary force during treatments at 100°C and 121°C, is not sufficient to entrap oil or ensure hydrophobic exposure [43]. Previous reports showed an increase in the OHC of RS3 cowpea starch produced using the autoclaving-cooling method [40]. These functional properties prompt the recommendation of RS3 Cilacap Breadfruit in the aspect of improving palatability and maintaining the taste of meat products.

TABLE III
FUNCTIONAL PROPERTIES OF CILACAP BREADFRUIT RESISTANT STARCH TYPE 3

Sample	Components	
	WAI (g/g)	OHC (g/g)
Native starch	0.52[f]±0.17	1.01[c]±0.57
100°C/24 h	3.22[b]±2.15	0.59[f]±1.28
100°C/48 h	3.47[a]±6.53	0.58[f]±0.80
121°C/24 h	3.11[c]±3.50	0.74[e]±1.48
121°C/48 h	2.83[d]±2.54	0.79[d]±1.72
140°C/24 h	1.95[e]±2.90	1.80[a]±1.27
140°C/48 h	1.94[e]±0.73	1.53[b]±3.90

Also, there is a possible application as a thickener in pudding and sauce.

C. Physical Properties

The physical properties were evaluated in the native and RS3 from Cilacap breadfruit, consist of X-ray diffraction in native starch and granule morphology in native and RS3.

1) X-ray Diffraction: This analysis aims to determine the crystal pattern in native starch. The results shown in Fig. 1 indicate the diffraction peak at 2 ϴ of about 5.6°-5.8°, 14°, 15°, 17°, 19°, 22°, and 23° (see arrows), confirming the presence of a type B crystal pattern [11],[47]. However, there are generally 4 types, including type A, B, C, and V [38], where A has characteristic peaks at 15°, 17°, 18° and 23° with double peaks at 17° and 18°, and are found especially in starch cereals. The Type B variety has characteristic peaks at 5.8°, 15°, and a single peak at 17°, usually found in tubers [11]. In addition, type C comprises a mixed pattern of A and B, with strong peaks around 15°, 16°, 18°, and 23°, and tend to be sourced from legumes. Moreover, the characteristic strong peaks of type V are around 14° and 19° [40]. Previous studies on the production of RS breadfruit starch after heat moisture treatment (120°C, 4 h) at various humidity (15, 20, 25, 30 and 35% w/w) showed changes in the X-ray diffraction pattern of native starch (type B crystals) to a combination of type A and B. In addition, HMT is one of the physical processes used to increase RS and improve the physicochemical and functional properties. This also ensures the movement of the dissociated double helix, the rearrangement and breakdown of the molecular chains, and the dissection of original hydrogen bonds [47]. Other research has shown a limited application of HMT treatment on cocoyam starch to the increase in diffraction intensity, as the crystal pattern is unaffected [68]. Moreover, the modification of native Cilacap breadfruit starch into RS3 possibly causes crystal changes, also observed in resistant starch produced through the autoclaving-cooling-storing method. This is an interesting area to conduct further research.

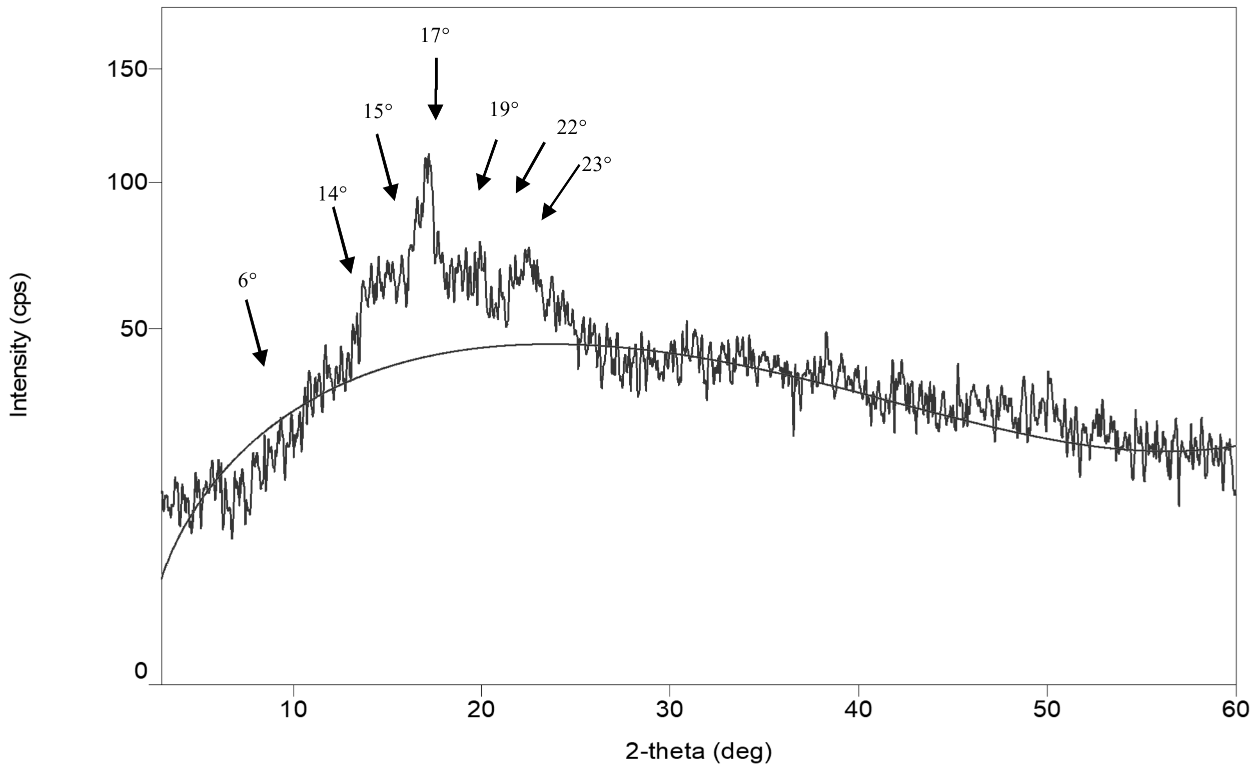

Fig. 1 X-ray diffraction characteristics of Cilacap Breadfruit

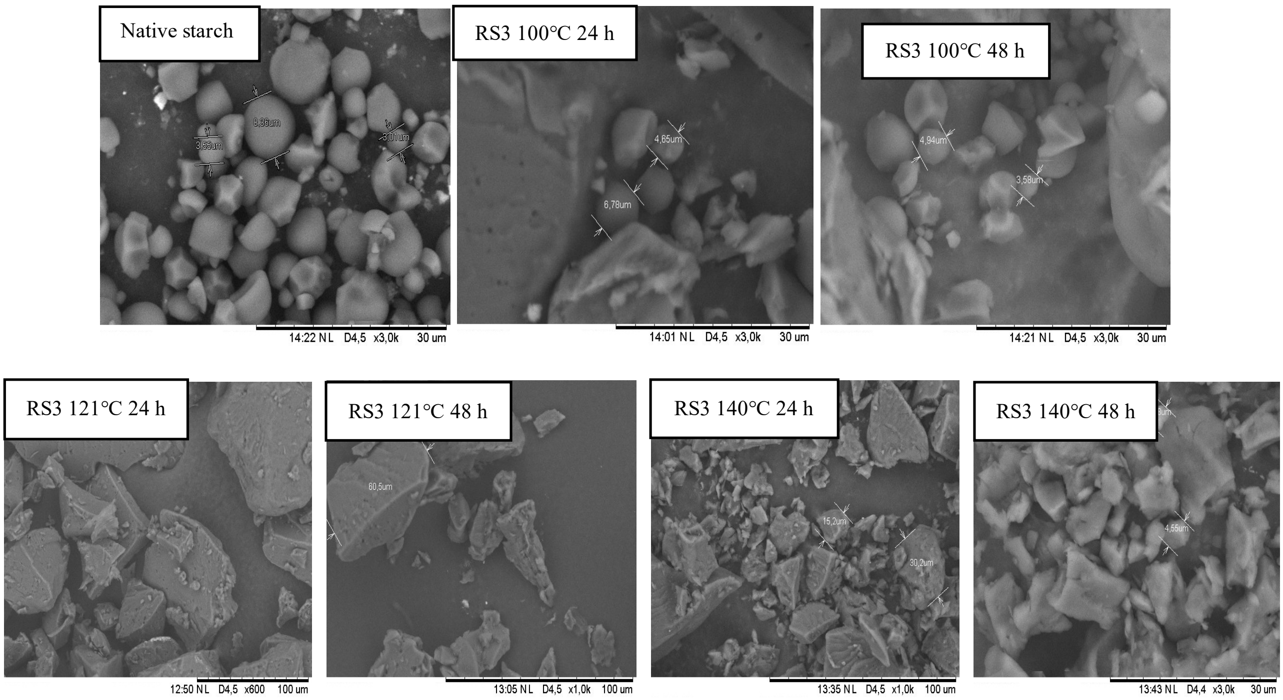

Fig. 2 Scanning electron micrographs of native starch and RS3 Cilacap breadfruit. The structure of native starch with a spherical and polyhedral shape, the structure of RS3 100°C still show some intact granular structure without change of form, RS3 121°C and 140°C, storage of 24 h and 48 h at 4°C, all granules are block-shaped with the size is non-uniform, compact, irregular shape

2) Granule Morphology: Fig. 2 shows the analysis results of starch granules, and the size for the native variety was categorized as small, within the range of 2.77-8.36 µm, alongside a spherical and polyhedral shape. These results are consistent with the evaluation outcome of rounded shape breadfruit samples originated from Ijokodo, Ibadan, Nigeria with a small average size of 4.34 µm, ranging from 2.25-8.42 µm [11]. Other reports on isolates from Madagascar possess an elliptical, spherical, and polyhedral shape, with a diameter of about 10 µm [47]. The diversity of granule structure is attributed to numerous factors, including agronomy, climate, processing conditions, and most importantly the variations in the amylose-amylopectin ratio [43]. The granule size also influences the sample physicochemical properties, as smaller forms instigate the leakage of more amylose molecules, leading to relatively lower pasting temperatures, compared to the larger granules [11].

Breadfruit RS3 produced at 100°C followed by cooling and storage at 4°C for 24 and 48 h show some intact granule structures without any modifications in form. However, some changes occurred in the shape of blocks, featuring non-uniform size, compact, irregular shape. Meanwhile, successive increases in autoclaving temperatures (121°C and 140°C), storage of 24 and 48 h at 4°C cause the disappearance of granule structure, and irregular structural formation. Also, all units demonstrate blocks of non-uniform size, compact, irregular shape, which is congruent with previous reports [40]. The breadfruit gelatinization process ensures amylose-amylopectin molecule recrystallization, characterized by the closer rearrangement towards each other through hydrogen bonding. Furthermore, re-composition is also possible, thus implicated in the formation of mixed crystals. This features the loss of amylopectin crystalline areas during heating, leaching of amylose molecules, and reassociation of granular starch-forming chains, during the autoclaving-cooling-storing process [69]. The modification of crystalline structure to be denser increases the starch molecule resistance towards the attack of digestive enzymes [43].

The results of research on the native and RS3 Cilacap breadfruit starch characteristics are expected to improve the understanding of the modification process, especially for samples obtained from Cilacap, Indonesia. The outcome of autoclaving, cold storage, and hydrolysis treatment is expected to contribute to developing new functional food ingredients. This includes the added benefits to consumer health, encompassing reducing glycemic index [70]. The consumption of RS3 by rats showed a significant decline in blood glucose levels in the first 120 minutes [65], hence the authorization by the US FDA for use in risk reduction, aimed at people with type 2 diabetes [23]. In addition, functional foods with a low glycemic index play an important role in preventing several degenerative diseases, comprising cancer, type II diabetes mellitus, obesity [40], and other degenerative illnesses.

There was a substantial decline in carbohydrate and starch content with subsequent storage over a longer period of 4°C (48 h), after autoclaving at the same temperature, compared to 24 h storage. In addition, extended duration (48 h) generally increases the antioxidant activity, alongside the insoluble and total fiber composition. This study, however, showed fluctuating treatment influences on the water, ash, fat, protein, and soluble fiber content of samples.

IV. Conclusion

Cilacap breadfruit starch has high carbohydrate content (91.40%) and is also rich in starch (82.85%), hence the possibility for application as an alternative source in the future. The manufacture of resistant starch through autoclaving method involved treatment at various temperatures (100, 121, and 140°C), followed by cooling and storage at 4°C for 24 and 48 h. The samples were subsequently exposed to acid hydrolysis, which significantly decreased the carbohydrate levels (from 91.40 to 91.19-87.88%) and starch content (from 82.85 to 82.36-77.82%). This outcome was augmented under a longer cold storage duration (48 h) despite similar autoclaving conditions ($p \leq 0.05$). This circumstance also caused an increase in the antioxidant activity (from 3.59 to 9.11%), insoluble (from 1.6 to 4.93%), and total food fiber (from 2.73 to 7.23), compared to samples reserved for 24 h. The Cilacap breadfruit starch autoclaved at 140°C and stored at 4°C for 48 h produced the highest antioxidant activity (9.11%). Also, all RS3 manufactured showed fluctuating values in the aspect of water, ash, fat, protein, as well as soluble fiber content. Moreover, native starch has a type B crystal pattern. The production of RS3 leads to increased WAI and OHC values. Furthermore, the granule structure of RS3 is lost after autoclaving at 121°C and 140°C, for 24, and 48 h. This results from the physical transformation into irregular-sized solids with block shapes. Hence, the yield from treatment with high gelatinization temperature possesses finer particle sizes, with the possibility of application to food products. This improves texture as well as color and also provides a better mouthfeel and flavor. In terms of soluble fiber composition, the physiological functions confer value as an ingredient in the formulation of functional foods to ensure beneficial health effects. Furthermore, the modified starch products also have commercial applications.

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