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The Role of Coconut Milk Ratio and Cooling-Reheating Cycle in Resistant Starch Type 5 of Buras as Indonesian Traditional Rice Cake

Muhammad Aditya Prawira, Yudi Pranoto*, Djagal Wiseso Marseno and Aisyah Mutiara Sabrina

Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Gadjah Mada University, Yogyakarta 55281, Indonesia

(*Corresponding author's e-mail: pranoto@mail.ugm.ac.id)

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Abstract

Some traditional carbohydrate-based foods in various Southeast Asian countries use coconut milk as one of their ingredients. One is a typical Indonesian rice cake called Buras. The presence of coconut milk can increase the resistant starch (RS) type 5 content in Buras because coconut milk contains fat. Another influential factor besides fat is cooling-reheating. This study aims to determine the effect of coconut milk ratio and cooling reheating on RS Buras. Four different ratios of coconut milk (Water: Coconut milk) were used for making Buras: 1:1, 2:1, 3:1 and 4:1. Increasing the ratio increased the RS content. The results showed that coconut milk ratio factors significantly increased the RS content ($p<0.05$). Buras R1 or Buras with a coconut milk ratio of 1:1 (100 mL water: 100 mL coconut milk) produced the highest RS content (30.55 %). Two cycles of cooling-reheating resulted in higher RS. Two cycles (S2) of cooling-reheating increased the RS content of the R1 Buras sample by 9.57 % and had the lowest digestibility at 180 min (18.94 %). The best-treated sample was R1 with two cooling-reheating cycles (R1S2). The coconut milk ratio also decreases starch digestibility. The best-treated sample was also R1S2 because it had the lowest digestibility. The low digestibility and high RS are due to the crystalline phase and the presence of an amylose-lipid complex, as shown by XRD and FTIR analysis. SEM analysis of the Buras with the best ratio (R1) showed a smooth, luminous granule surface surrounded by fat.
Keywords: Resistant starch, Coconut milk ratio, Cooling-reheating, Starch digestibility, Buras

Introduction

Resistant starch (RS) is an alternative to managing degenerative diseases such as diabetes mellitus. Diabetes is caused by consuming products high in Glycemic Index or carbohydrate-containing foods' high digestibility rate. Consumption of RS can be considered in addition to the use of drugs or insulin injections. Consuming RS can suppress the increase in blood glucose by inhibiting the digestibility of starch in the body. RS is a starch fraction that cannot be hydrolyzed into D-glucose in the small intestine but is digested through the colon microbe's fermentation [1]. RS consists of several types based on the processing method [2]. Type 1 RS is a naturally occurring starch in plant cells or food matrices, including grains and cereals. Type 2 RS refers to starch naturally resistant to digestion, typically in crystalline granules. Type 3 RS is starch that experiences retrogradation after being subjected to a heating process followed by cooling. Type 4 RS encompasses starch that has been chemically modified, such as through esterification or cross-linking. Type 5 RS is starch that binds with lipids to form starch-lipid complexes, where amylose interacts with fatty acids or fatty alcohols to create a single helix structure. Type 5 RS is also called lipid-amylose complexed starch. The amylose-lipid complex is formed when the amylose released during starch gelatinization forms a single helix with a suitable ligand. These ligands can be fatty acids, monoglycerides or lysophospholipids [3] that enter the hydrophobic amylose cavity, forming complexes resistant to digestive enzymes. The

mechanism by which amylose-lipid complexes are susceptible to digestive enzymes compared to free amylose is because these complexes resist the extent of granule expansion. Therefore, it is difficult for enzymes to reach the inside of starch granules [4]. Some foods have RS5 or amylose-lipid complexes because they contain coconut milk.

About 25 % of the coconut produced globally is consumed as coconut milk [5]. Some carbohydrate foods in some Southeast Asian countries use coconut milk as an ingredient. One is a food made of rice combined with coconut milk found in some Southeast Asian countries. Examples are Suman from the Philippines, Mango Sticky Rice from Thailand, Nasi Lemak from Malaysia and Nasi Uduk from Indonesia. Nasi Uduk has recently been investigated as a local Indonesian food that contains RS5. Nasi Uduk is rice cooked with coconut milk, which has a savory taste. The use of coconut milk can affect the RS content of nasi Uduk. This is caused by the RS5 existence or complex of amylose-lipid due to the binds of amylose from rice and fatty acids from coconut milk. Research reported that nasi Uduk has an RS content of 5.35 and 8.16 % [6,7], both were classified as high [8]. A similar product that potentially contains RS5 is Buras. Buras is a local Indonesian food that originated in South Sulawesi Province. It is made from rice cooked with coconut milk until gelatinized. Buras have a structure resembling rice cakes and a sticky texture. Studying Buras regarding RS5 content will be very important because research on Buras has never been found before, has the potential as a food that can manage diabetes, and at the same time promotes local culture which is expected to be beneficial for health. However, the high or low levels of RS produced in Buras are influenced by several factors.

Several factors affect the RS type 5 formation, such as the starch type, lipid type, lipid ratio and the conditions of amylose-lipid complex formation [9]. Buras is gelatinized more completely, as seen from its denser form than Nasi Uduk, so the RS content may be higher. However, the impact of different coconut milk ratios on the RS content of Buras is not yet known. Other than the coconut milk ratio, processing methods such as cooling-heating may influence RS formation in Buras. Storing food in a refrigerator cold and reheating it is a common practice in households almost all over the world, especially in Asian countries. Cooling-reheating is known to increase RS levels, including with some cycles. Rice cooled after cooking and then reheated after 12 and 24 h could increase RS levels by 2 and 2.5 times higher [10]. Cooling-reheating cycles also have not been known to increase the RS content in Buras. Therefore, this study evaluated the physicochemical properties and digestibility of RS type 5 in Buras treated with different coconut milk ratios and cooling-reheating cycles. This research is also expected to provide new insights to society regarding the health benefits of Buras because it contains resistant starch and the potential to manage degenerative diseases such as diabetes mellitus.

Materials and methods

Materials

Rice var. Setra Ramos (also called IR 64) was selected because of its high amylose content, salt as a flavor enhancer, bay leaf as an aroma enhancer, and banana leaf used as wrapping material for cooking. All these ingredients are obtained from the local market in Sleman, Yogyakarta, Indonesia. Commercial coconut milk (Kara, Indonesia) and drinking water (Le Minerale, Indonesia) were obtained from the convenience store in Sleman, Yogyakarta, Indonesia.

Preparation of coconut milk with different ratio

The ratios used were determined from the general recipe of the society and Buras traders, who overall used a ratio of 1:1 (100 mL water: 100 mL coconut milk) or 2:1 (200 mL water: 100 mL coconut milk). In contrast, 3:1 and 4:1 is used as additional variations. Therefore, coconut milk was made with each of 4 ratios and measured using a measuring cup with these ratios (v/v): R1 = 1:1 (100 mL water: 100 mL commercial coconut milk), R2 = 2:1 (200 mL water: 100 mL commercial coconut milk), R3 = 3:1 (300 mL water: 100 mL commercial coconut milk) and R4 = 4:1 (400 mL water: 100 mL commercial coconut milk). Buras is then cooked using each of these different ratios.

Preparation of Buras

A 100 g of rice was washed 2 times using tap water (300 mL). The 100 g rice was then mixed with 200 mL coconut milk from each ratio (R1, R1, R3 and R4). Then, 1 bay leaf and 1 g of salt were also added. The mixture was then cooked using a rice cooker (Cosmos

CRJ-6123, Indonesia) ± 20 min until automatic shutoff. The cooked rice was cooled at room temperature for 10 min. A 50 g of cooked rice was taken and then wrapped in banana leaves into pre-gelatinized Buras sized 8×5×2 cm^3 in a rectangular shape. These pre-gelatinized Buras were tied using plastic rope and then boiled for 60 min in boiling water using a stove (Modena FC 3955, Modena Technology Ltd., Italy). After being cooked and gelatinized, the Buras were cooled at room temperature for 1 h.

Sample preparation

The cooling-heating treated Buras were cooled in the cooling room (4 °C) for 24 h and reheated using a microwave oven (Electrolux EMM2308X, Electrolux, Indonesia) at medium heat intensity (450 W output; 80 - 100 °C) for 5 min. The Buras (±100 - 125 g per 1 Buras) are cut into dice-sized pieces using a knife and then placed into plastic cups with a diameter of 30 mm and height of 50 mm. The dice-sized Buras were dried in a freeze dryer (Labconco FreeZone 2.5 L -50 °C Benchtop, USA) at −47 °C for 24 h with a high vacuum pressure of 0.099 mbar. After the drying process, the Buras were ground using a pestle and mortar into a fine powder. The Buras powder was then filtered using an 80-mesh sieve (0.180 mm particle size) to ensure uniform particle size and suitability for further analysis.

Resistant starch content analysis

Pre-treatment Buras starch removed its fat first, referring to the defatting method [11]. A 5-gram sample was measured into a 45 mL falcon tube. The sample was added with 50 mL of n-hexane. The mixture was heated on a hot plate at 40 °C and stirred with a magnetic stirrer for 1 h. Following this, the sample was centrifuged at 2,300 rpm at room temperature (27 - 30 °C) for 10 min. This centrifugation was repeated 4 times to ensure that undesirable fat in the sample was completely removed. Finally, the sample was left to dry on aluminum foil at room temperature for 24 h until the solvent had evaporated completely.

Analysis of resistant starch content was performed according to the method of Goñi *et al.* [8]. A 25 mg flour sample was put into a 15 mL falcon tube, and 2.5 mL of KCl-HCl buffer pH 1.5 was added. The mixture was then homogenized using a vortex after adding 50 µL of pepsin (Sigma P7000, Sigma-Aldrich Inc., US) solution (0.1 g pepsin powder/ 1 mL KCl-HCl buffer). It was heated for 60 min at 40°C while constantly shaken in a water bath shaker (Labodam LD-LWBS-A10, UK) with orbital motion and then cooled at room temperature. Then, the mixture was added 2.25 mL of 0.1 M Tris-maleate buffer solution pH 6.9 and 250 µL of α-amylase (Sigma A3176, Sigma-Aldrich Inc., US) solution (80 mg α–amylase powder/2 mL Tris-maleate buffer). For 16 h, the mixture was continuously shaken in the water bath with orbital motion at 37 °C. After that, the mixture was centrifuged at 3,000 rpm for 15 min at room temperature (27 - 30 °C), then the supernatant was disposed of. The remaining buildup was washed with 10 mL of distilled water, centrifuged once more with the same settings as the previous one, and the supernatant was disposed of. After adding 0.75 mL of 4 M KOH solution and 3 mL of distilled water to the residual, it was vortexed. For 30 min, the mixture was shaken constantly while incubated at 37 °C in a water bath. Then 1,375 mL HCl 2 M and 0.75 mL of buffer sodium acetate pH 5.2 were added to the mixture. The mixture was then added 20 µL of aqueous amyloglucosidase enzyme from *Aspergillus niger* (Sigma A7095, Sigma-Aldrich Inc., US) and vortexed, then incubated again in a water bath shaker with constant orbital shaking at 60 °C for 45 min. The sample was then centrifuged at 3,000 rpm for 15 min at room temperature (27 - 30 °C), and the supernatant was removed and transferred to a 25 mL measuring flask. After adding 10 mL of distilled water to the residue, the centrifuge was run again with the same settings as the previous one. The earlier supernatant was then combined with the new one. Aquadest was added until it reached the 25 mL calibration limit of the measuring flask. A standard curve of glucose solution was prepared (20 - 100 ppm) from the GOD-FS kit (Ref 1 2500 99 83 021, DiaSys Diagnostic Systems GmbH, Germany). A 0.5 mL sample solution was put into a test tube, while 0.5 mL of distilled water was used as a blank. A 1 mL GOD solution was added to the sample solution and blank, then vortexed. The mixture was then incubated in a water bath shaker at 37 °C for 30 min. The absorbance of the sample was measured using a spectrophotometer (Shimadzu UV-1280, Japan) at a wavelength of 500 nm (5 min after incubation). Resistant starch content was calculated using the following equation:

$$\text{RS (\%)} = \frac{\text{Glucose}\left(\frac{\text{mg}}{\text{mL}}\right) \times \text{DF} \times 0.9}{\text{Sample weight (mg)}} \quad (1)$$

Glucose (mg/mL) refers to the glucose concentration derived from the calculation of sample absorbance using the glucose standard curve equation, DF is the dilution factor obtained from the total volume of the sample solution, 0.9 is the conversion factor from glucose to starch, and sample weight (mg) is the mass of the starch sample that was used for analysis.

***In vitro* starch digestibility**

The digestion of starch has been carried out through an *in vitro* procedure referred to Goñi *et al.* [12] with 3 repetitions to enhance the reliability of the results. A 10 mL KCl-HCl was added to a 50 mg sample and vortexed for 2 min. Then, in a water bath shaker (Labodam LD-LWBS-A10, UK), 0.2 mL of pepsin enzyme solution (0.2 g pepsin powder/ 2 mL KCl-HCl) was added, and the mixture was incubated for 1 h at 40 °C. The volume was diluted to 25 mL by adding 14.8 mL of Tris-Maleate buffer pH 6.9 to each sample. Samples were added with 5 mL of α-amylase solution (40 mg α-amylase powder/ 1 mL Tris-Maleate buffer) (Sigma A3176, Sigma-Aldrich Inc., US) and then incubated at 37 °C in a water bath shaker with orbital motion for 60 min. A 0.5 mL sample of aliquots was taken from each tube every 30 min from 0 to 180 min. The aliquot was transferred to a tube and heated at 100 °C above a hot plate for 5 min to deactivate the enzyme, then allowed to cool until the incubation period was complete. Each aliquot was filled with 200 µL of 0.4 M sodium acetate buffer at pH 4.75. After adding 30 µL of aqueous amyloglucosidase enzyme from *Aspergillus niger* (Sigma A7095, Sigma-Aldrich Inc., US) to hydrolyze the digested starch into glucose, the mixture was shaken continuously with orbital shaking for 45 min at 60 °C. Aliquot was added with 1.5 mL of GOD-FS (Ref 1 2500 99 83 021, DiaSys Diagnostic Systems GmbH, Germany). For 20 min, the sample was incubated at 50 °C. A spectrophotometer (Shimadzu UV-1280, Japan) was used to detect the absorbance of samples at 510 nm wavelength. The rate of starch digestion was determined based on the percentage of total starch hydrolyzed at various time intervals (30, 60, 90, 120, 150 and 180 min) using the following equation:

$$\text{Total starch hydrolysis (\%)} = \frac{\text{Release glucose weight}\left(\frac{\text{mg}}{\text{mL}}\right) \times 0.9}{\text{Sample weight (mg)}} \times 100 \quad (2)$$

Released glucose weight (mg/mL) represents the glucose released during enzymatic hydrolysis. This is measured by determining the sample absorbance at 30-minute intervals (0 - 180 min) and calculating the glucose concentration using a glucose standard curve. The 0.9 was used to convert the glucose weight into its starch equivalent. Sample weight (mg) refers to the initial mass of the starch sample used for enzymatic hydrolysis. The 100 was a scaling factor applied to express the result as a percentage, indicating the proportion of starch hydrolyzed relative to the total sample weight.

Fatty acid composition

Pre-treatment The methylation procedure referred to AOAC Official Method 969.33 [13] with little modification. A 0.05 g coconut milk sample was measured with an analytical balance (Fujitsu FS-AR210, Japan). It added 400 µL of 0.5 M methanolic NaOH (0.1 g NaOH/ 5 mL methanol), vortexed, heated for 10 min at 50 °C, and cooled. After that, 6.5 mL of methanolic BF3 (2.5 mL BF3 14 %/ 12.5 mL methanol) was added and heated for 2 min. Then, 1 mL of distilled water and 5 mL of n-hexane (pro analysis grade, Supelco) was added. The mixture was vortexed and allowed to stand for several mins until 2 layers were formed. The top layer was taken for GC analysis.

Gas Chromatography (GC) analysis of the methylated coconut milk sample was conducted using a gas chromatograph (Shimadzu GC-2010 Plus) equipped with an FID (Flame Ionization Detector). The separation was achieved using a STABILWAX-DA capillary column (30 m length; inner diameter 0.25 mm and film thickness 0.25 µm). A 1 µL sample was injected in a split mode (split ratio 40:1) into the GC apparatus. The injector temperature was set at 240 °C, and the carrier gas used was helium, operated in pressure control mode with a column flow rate of 0.81 mL/min, a linear velocity of 25.2 cm/s, and a total flow rate of 36.1 mL/min.

The column oven temperature program was as follows: An initial temperature of 150 °C was held for 0.00 min, followed by an increase of 8.0 °C/min to 200 °C, which was maintained for 19.00 min. The

temperature was then further increased at 8.0 °C/min to 220 °C with no hold time, resulting in a total program time of 27.75 min. Helium was also used as the makeup gas at a flow rate of 30.0 mL/min. Hydrogen and air were supplied at flow rates of 50.0 and 400.0 mL/min, respectively.

The GC chromatogram results were analyzed using LabSolutions (Shimadzu) software to integrate the peak area of each identified fatty acid based on certified reference material FAME Mix C8-C24 standard (Product No.: CRM18918, Supelco, Sigma-Aldrich). Quantification was performed using the peak area normalization method, where the peak area of each fatty acid was compared against the total peak area of all detected components. This procedure yields the relative percentage of each fatty acid in the sample.

X-ray diffractometry (XRD)

The crystalline structures of the amylose-lipid complexes in Buras were analyzed using an X-ray diffractometer (Bruker AXS D8 Advance Eco, Germany). Cu-Kα radiation was used with 40.0 kV and 25.0 mA operation. The diffractograms of each sample were collected within the range of 5 to 50 ° (2θ), with a 0.020 ° step size. The peak that appears can determine the phase of the sample. The measurements were done without repetition. This is because the XRD method does not focus on evaluating sample variability, so it does not require statistical analysis that utilizes repeatability. In addition, XRD produces highly precise and reproducible data when standardized procedures are followed correctly.

Fourier transform infrared (FTIR) spectroscopy

The FTIR spectra of the Buras were recorded using a Nicolet iS10 FTIR spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). Prior to analysis, the samples were combined with KBr powder (1:100, w/w) in a quartz mortar. Samples were then pressed in a nut between two tightened bolts, resulting in transparent pellets for measurement. The spectra were acquired at 4 cm^{-1} resolution and 4,000 to 500 cm^{-1} range. Repeated measurements were not conducted as the focus was solely on identifying absorption bands corresponding to specific functional groups rather than obtaining values requiring reliability or standard deviation analysis. Additionally, FTIR spectra are known for their high stability and reproducibility, rendering repeated measurements unnecessary as they would not provide significant additional information.

Scanning electron microscopy (SEM)

The granule morphology of Buras was observed using a JSM-6510 Series (Japan) Scanning Electron Microscope. A metal stub was used to mount the samples using black conductive tape adhered to the top before observation. Each sample was coated with gold by a sputter coater under vacuum conditions. The surface structures of Buras granules were observed at a resolution of 1,000× with a 10 kV accelerating voltage.

Statistical analysis

Descriptive analysis was used to describe the fundamental characteristics of the research subjects. Sample repetition and analysis repetition were done in triplicate. IBM SPSS Statistics 25 software (IBM, USA) was utilized to measure the differences between properties using the 1-way analysis of variance (ANOVA) study with a p-value ≤ 0.05 significant level. The experimental data was plotted using OriginPro 2024b (Learning Edition) software (Origin Lab Corporation, Northampton, MA, USA).

Results and discussion

Fatty acid composition

Composition analysis of fatty acids was conducted to determine the percentage of fatty acids in 100 % commercial coconut milk and various ratios of coconut milk to water (R1, R2, R3 and R4). This was done to justify whether the high or low amount of fatty acids in coconut milk is directly proportional to the high or low RS formed in Buras. The types and composition of fatty acids in coconut milk are listed in **Table 1**. The coconut milk ratio factor influenced the amount of detected fatty acids. It is proven that the more concentrated the coconut milk ratio or the higher the fatty acid content, the higher the formation of RS (**Table 2**).

Table 1 Fatty acid composition of different coconut milk ratio.

Ratio	Coconut milk (%)							
	Caprylic (C8:0)	**Capric (C10:0)**	**Lauric (C12:0)**	**Myristic (C14:0)**	**Palmitic (C16:0)**	**Stearic (C18:0)**	**Oleic (C18:1n9)**	**Linoleic (C18:2n6)**
100 % coconut milk	9.40 ± 0.47^{c}	9.15 ± 0.14^{c}	80.72 ± 2.60^{c}	32.10 ± 2.74^{c}	15.39 ± 1.75^{c}	6.43 ± 1.01^{c}	7.76 ± 0.84^{c}	1.38 ± 0.23^{b}
R1	3.71 ± 0.92^{b}	3.72 ± 1.22^{b}	34.71 ± 11.49^{b}	14.10 ± 5.13^{b}	8.19 ± 0.83^{b}	3.17 ± 0.80^{b}	4.76 ± 0.31^{b}	1.12 ± 0.42^{b}
R2	2.17 ± 0.12^{a}	2.22 ± 0.29^{a}	21.18 ± 3.31^{ab}	8.60 ± 1.66^{ab}	4.13 ± 0.87^{a}	1.79 ± 0.36^{ab}	2.17 ± 0.54^{a}	0.30 ± 0.00^{a}
R3	1.63 ± 0.02^{a}	1.80 ± 0.04^{a}	17.59 ± 0.49^{a}	7.38 ± 0.11^{ab}	3.69 ± 0.29^{a}	1.63 ± 0.08^{ab}	1.95 ± 0.28^{a}	0.48 ± 0.10^{a}
R4	1.40 ± 0.07^{a}	1.36 ± 0.09^{a}	13.60 ± 0.57^{a}	5.61 ± 0.38^{a}	2.72 ± 0.36^{a}	1.25 ± 0.14^{a}	1.58 ± 0.38^{a}	0.33 ± 0.12^{a}

Values from triplicate calculations are presented as mean ± standard deviation. Different letters in the same row indicate significant differences ($p < 0.05$).

It is also known that the more diluted the commercial coconut milk to water (R1 to R4), the lower the percentage of all types of fatty acids detected. This indicates that the addition of water to fat results in hydrolysis reactions to form free fatty acids. The formation of free fatty acids in coconut milk can reduce the amount of fatty acids available in triglycerides. Side *et al.* [14] stated that the formation of free fatty acids can occur due to a certain amount of water, enzymes or microorganism activity. The mechanism is when the oxygen from the hydroxyl group (−OH) of water attacks the electrophilic carbon on the ester group (−O−R) of triglycerides. This interaction leads to the breakdown of the ester bond, resulting in the release of free fatty acids and the formation of glycerol as a by-product. Therefore, although the total amount of fatty acids remains the same, they now exist in the form of free fatty acids and not as triglycerides.

The fatty acid content of coconut milk is dominated by saturated fatty acids, especially lauric acid, reaching 80.72 %. This amount is 46.73 % higher than reported by Karunasiri *et al.* [15], who also used commercial coconut milk. The difference in the amount obtained may be due to differences in coconut varieties, harvest seasons and coconut milk extraction methods each industry uses.

Effect of coconut milk ratio on resistant starch content of Buras

RS5 is a resistant starch resulting from complexation between amylose and lipids that occurs due to gelatinization or retrogradation, this makes it indigestible by the digestive enzyme α-amylase. The mechanism of RS5 formation begins when gelatinization causes fatty acids to enter the hydrophobic cavity of free amylose, forming a stable interaction between amylose and lipids, known as RS5. This structure tends to be hydrophobic, so it is resistant to digestive enzymes that work in a hydrophilic condition. According to Okumus *et al.* [4], the resistance of amylose-lipid complexes to enzyme digestion is due to these complexes reducing the extent of granule swelling, making it difficult for enzymes to reach the interior of the starch granule. In addition, lipid concentration is one factor that potentially affects RS5 in Buras. Differences in the coconut milk ratio can influence the amylose-lipid complex, so the RS content can increase or decrease, as shown in **Table 2**.

R1 obtained the highest RS content (30.55 %) compared to the other treatments (**Table 2**). This RS amount, according to Goñi *et al.* [8] is classified as very high (> 15 %). The addition of coconut milk in R1 Buras increased RS levels in R0 to 14 %. This shows that the RS content increases along with the higher amount of coconut milk added. These findings are related to 2 similar studies by Liu *et al.* [16]; Chumsri *et al.* [17], which adjusted the concentration of fatty acids. Both studies observed RS content increased as the number of fatty acids added increased. The highest RS content, 39.94 % [16], was achieved by adding 8 % lauric acid to corn starch, while 80.78 % [17] was obtained by adding 7.5 % butyric acid to rice starch. Both were classified as very high RS levels (> 15 %) [8]. Align with both previous researches, the increased coconut milk ratio

(R4 to R1) leads to a higher formation of resistant starch (RS) in Buras. This increase is attributed to the formation of more abundant amylose-lipid complexes. Additionally, the increase in fatty acid content is not only due to their association with amylose but also their ability to coat the surface of Buras granules. Consequently, water mobility into the granules is disrupted, inhibiting starch gelatinization [17].

Table 2 Resistant starch content of Buras with different coconut milk ratio.

Ratio	Resistant starch content (%)
R0	26.80 ± 0.38^{b}
R1	30.55 ± 1.65^{c}
R2	27.17 ± 0.48^{b}
R3	24.55 ± 1.01^{a}
R4	23.05 ± 0.21^{a}

Values from triplicate calculations are presented as mean ± standard deviation. Different letters in the same row indicate significant differences ($p < 0.05$).

Buras without coconut milk addition (R0), although not having amylose-lipid complex or RS5, had higher RS content (26.80 %) compared to Buras with R3 (24.55 %) and R4 (23.05 %). This is attributed to retrogradation, which is the formation of crystalline structures in starch due to the cooling process after starch is gelatinized after cooking. This retrograded starch, classified as RS3, is formed through the recrystallization of several single chains (amylose) to form a double helix (amylopectin) through strong hydrogen bonding, thus increasing crystallinity and RS content [18]. The RS content of R0 is higher than that reported in similar research using the cooked rice model conducted by Anugrahati *et al.* [6] at 1.25 %; Pangastuti and Permana [7] at 1.36 %.

The higher RS content is due to the contribution of the physical structure of the cooked ingredients. Buras has a banana leaf structure that encloses the starch, preventing the movement of amylose molecules that escape into water. This causes the gelatinization of Buras to be more complete than nasi Uduk, thus triggering a more optimal retrogradation event. The polyphenol content of banana leaves is also thought to migrate and bind to the amylose of Buras, thus contributing to the increase of the RS content. Thus, further research is needed to explain differences in the RS content of products with different structures and the contribution of other ingredients to increase the RS content.

***In vitro* starch digestibility**

Starch digestibility was conducted to determine the rate of digestible starch from 0 to 180 min. Starch digestibility is also related to RS content. Starch that could not be hydrolyzed after 120 min was categorized as RS. In addition, samples that have the lowest digestibility are indicated high in RS content. *In vitro* starch digestibility uses several enzyme reagents to hydrolyze Buras starch, including pepsin, α-amylase and amyloglucosidase. Pepsin is used to hydrolyze the remaining protein, while α-amylase and amyloglucosidase work synergistically to digest the starch fraction in 2 stages [19]. First, α-amylase randomly cleaves substrate molecules on the granule surface, generally 1,4-glycosidic α-bonds to produce oligosaccharides (such as maltose and dextrin), which are more easily digested by other enzymes such as amyloglucosidase. Furthermore, amyloglucosidase breaks down oligosaccharides by hydrolyzing both α-1,4 and α-1,6-glycosidic bonds, making it easier for α-amylase to digest the remaining groups. The hydrolysis of Buras starch with all ratios is shown in **Figure 1**.

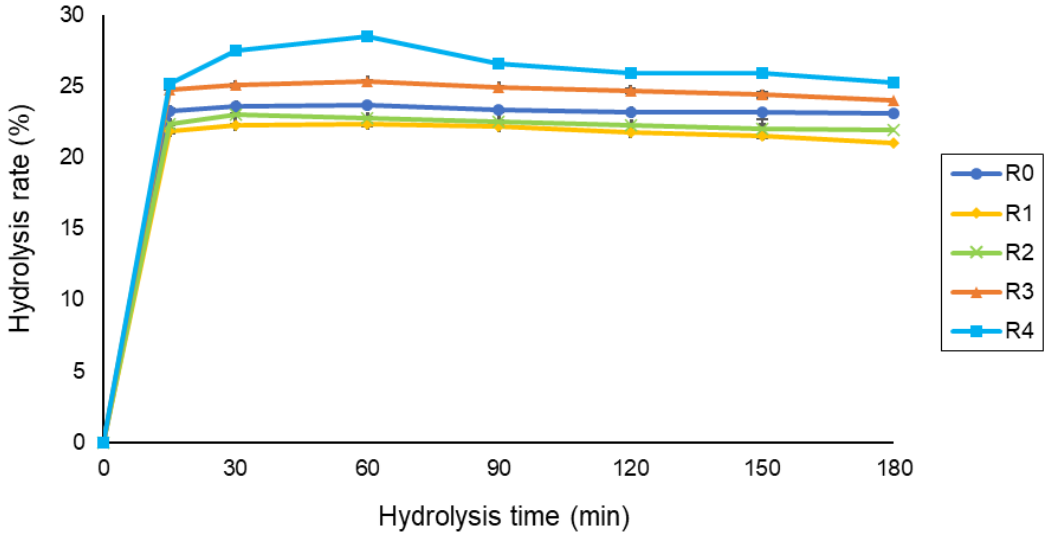

Figure 1 Enzymatic hydrolysis of Buras starch with different coconut milk ratios, including R1 (100 mL water: 100 mL commercial coconut milk), R2 = 2:1 (200 mL water: 100 mL commercial coconut milk), R3 = 3:1 (300 mL water: 100 mL commercial coconut milk) and R4 = 4:1 (400 mL water: 100 mL commercial coconut milk).

The results show that the digestibility of all samples decreased after 60 min. R1 sample had the lowest hydrolyzed starch with an amount of 21 % at 180 min, while R4 was higher than the other samples at 25.26 %. These results have a precise relationship with the increased resistant starch content (**Table 2**). The decrease in digestibility was due to a large amount of complex formation between starch and fatty acids [20], which indicated an amylose-lipid complex or RS5. This shows that increasing the proportion of coconut milk can alter starch digestibility by introducing additional lipids or fatty acids to bind to amylose. These changes increase the stability of the amylose-lipid complex and decrease or inhibit starch digestibility.

Starch without coconut milk addition (R0) had less hydrolyzed starch than R3 and R4. This digestibility inhibition was triggered by starch retrogradation, presumably when the Buras were cooled at room temperature after cooking. Starch retrogradation involves recrystallizing amylose chains and forming double helices tightly bound by hydrogen bonds [21]. The Buras with the highest RS content (**Table 2**) and the lowest digestibility (**Figure 1**) were further processed for repeated cooling-heating cycles. Selected Buras with repeated cooling-heating cycles were further analyzed for RS content and digestibility.

Morphology analysis

The Buras that was previously freeze-dried were then grounded. The flour was analyzed for granule morphology using a Scanning Electron Microscope (SEM). Granule morphology was observed for Buras without coconut milk addition (R0) and with coconut milk addition (R1, R2, R3 and R4). SEM images of R0 (**Figure 2(a)**) show a tough surface and a disintegrated structure. This is thought to be due to the gelatinization and degradation of starch due to high pressure, intense heat, and shear forces [22]. A slightly porous surface is also visible. The porous microstructure is due to the cooking process, which triggers the gelatinization effect on the starch granules [23].

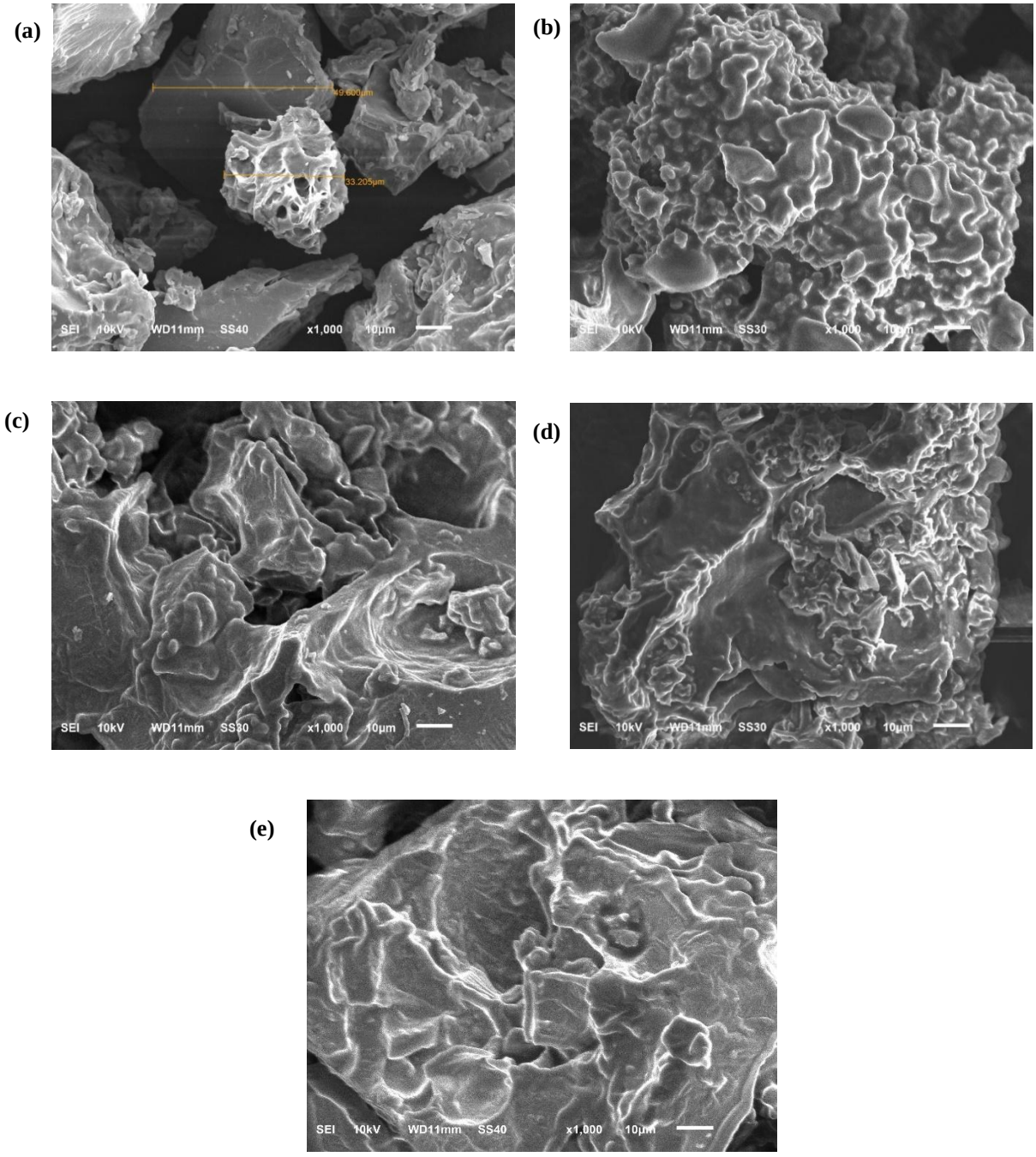

Figure 2 Scanning Electron Microscope (SEM) images of Buras without coconut milk addition (R0) and with coconut milk addition in different ratios (R1 - R4).

SEM images of amylose-lipid complexes (**Figures 2(b) - 2(e)**) show starch granules disintegrated and aggregated with fat. The addition of coconut milk led the Buras granule to form an irregular structure. This is also due to the gelatinization effect of heat treatment during cooking. Gelatinization causes the granules to melt and aggregate glucan chains, resulting in a stretched granule structure [24]. Heat treatment can enhance complexation or esterification reactions, especially when using gelatinized starch [25]. However, it has been suggested that some fatty acids cannot penetrate the starch granules to form amylose-fatty acid complexes but instead form a complex layer around the starch granules [26]. This also resulted in the luminous surface of all Buras starch with coconut milk addition.

Crystalline structure

Starch crystallinity was analyzed to determine the crystallinity phase of Buras starch. The degree of peak (with diffraction angle of 2θ) and its intensity illustrated

the sample phase, whether it is crystalline or amorphous. The sample is defatted first to remove the lipid that covers the Buras starch particles, preventing it from interfering with the detector's absorption. The defatting leaves the amylose-lipid complex or the fat that only binds to the starch. According to Tansman *et al.* [27], defatted samples produce diffractograms that are less noisy and easier to use when performing chemical analysis.

Starch-lipid complexes typically arrange V-type crystals and show unique peaks around 7, 13 and 20 ° [28]. The V-type crystal is defined as a structure of amylose that is single-helix and left-handed, with a typical ligand such as fatty acid occupying the helical cavity. **Figure 3** shows that all samples exhibit peaks at 17 and 20 °. The 17 ° peak is attributed to the presence of amylopectin recrystallization. The 20 ° peak is associated with the well-formed V-type structure resulting from complexation with compounds that have polar and non-polar partitions, usually fatty acids [17]. The additional peak at 21 ° correlated to the existence of amylose-lipid complex crystalline structure in the granules [29].

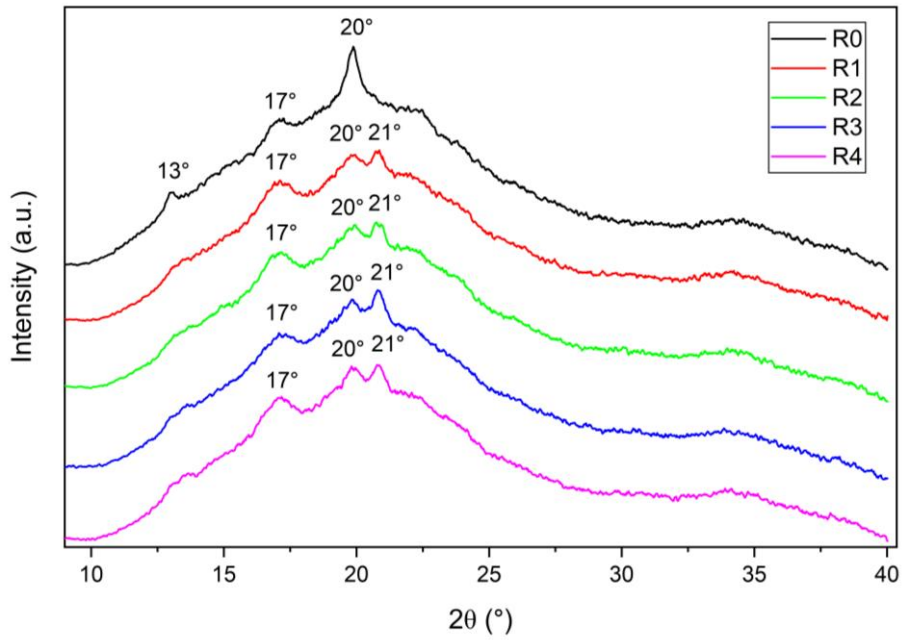

Figure 3 XRD pattern of Buras starch without coconut milk (R0) and with coconut milk addition in different ratios (R1 - R4).

The Buras sample without coconut milk addition (R0) showed peaks of 13 and 20 ° even though there was no lipid contribution. These 2 peaks indicate the effect of cooling-reheating. The 13 and 20 ° peaks resulted from the twice-treated autoclaving-retrogradation of native starch, which are the unique peaks of a V-type pattern [30]. Based on this study, both peaks are due to heating followed by post-cooking cooling, which causes retrogradation in R0.

The crystallinity of starch is not only determined by the peak that appears, but also the sharpness of the peak. V-type crystals that have been known from the peaks that appear can be divided into 2 amorphous forms, namely complex form I and complex form II. Complex form I has a wide peak characteristic, while complex form II has a sharp peak [16]. This type II complex is also called the crystalline phase [31].

Based on this observation, the phase of all Buras samples can be classified as semi-crystalline. This is because the control sample (R0) has 2 of the 3 total peaks typical of V-type crystals, there are 13 and 20 °, but only 20 ° had a sharp peak. The samples with the addition of coconut milk (R1, R2, R3 and R4) all had wide peaks, but only one typical peak that describes V-type crystals, which is 20 °.

The crystallinity decreases as the fatty acid chain length increases. This is thought to be because the C-H groups of fatty acids disrupt the bonding of amylose

with lipids, causing the formation of a less regular crystal structure [32]. Based on this, Buras with coconut milk addition (R1 - R4) may be less effective at forming an ordered crystal structure due to the presence of fatty acids compared to control (R0).

FTIR analysis

FTIR analysis was conducted to qualitatively identify functional groups present in the sample, as the technique reliably detects the molecular vibrations and chemical bonds of interest. The determination is based on the absorption bands from the detected sample. The absorption bands have wave numbers that describe certain functional groups. FTIR analysis of the Buras sample is useful to confirm the amylose-lipid bond formed by the interaction between coconut milk's fatty acids and rice starch. **Figure 4** displays the FTIR spectra of each Buras without (R0) and with coconut milk addition (R1 - R4).

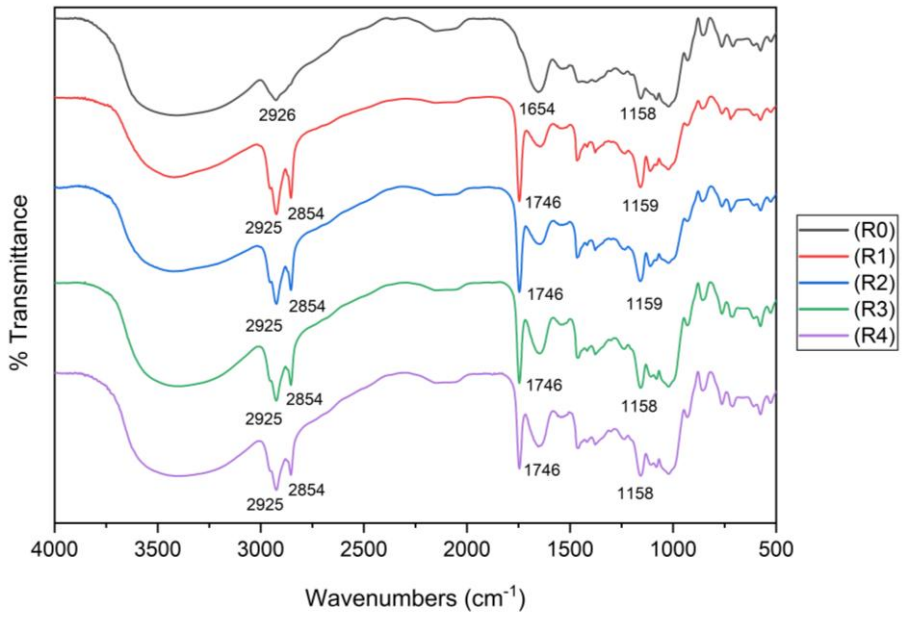

Figure 4 IR Spectrum of Buras starch without coconut milk (R0) and with coconut milk addition in different ratios (R1 - R4).

All Buras samples with coconut milk addition (R1, R2, R3 and R4) exhibit 2,925 and 2,854 cm^{-1} absorption bands, corresponding to the stretching vibrations of methyl ($–CH_3$) or methylene ($–CH_2$) groups of fatty acids [33]. These peaks are considered key indicators of the presence of an amylose-lipid complex. The formation of this complex is associated with methyl groups (–CH) found in lipid molecules, which interact with amylose through hydrophobic interactions. Methyl groups are the main indication of the presence of amylose-lipid complexes due to their nonpolar nature and are highly reactive to hydrophobic interactions compared to other groups. This facilitates interpretation suggested as a direct indicator of amylose-lipid complex formation.

The formation of the 2,925 and 2,854 cm^{-1} absorption bands was strongly related to the increase in RS content and decrease *in vitro* starch digestibility, where the highest RS content (30.55 %) and the lowest *in vitro* starch digestibility (21 %) at 180 min were observed in R1 (**Table 2**; **Figure 1**). As evidenced by the sharp FTIR absorption band in sample R1 although similar to samples R2, R3 and R4, this indicates that the amylose-lipid complex formation is more stable in R1. The formation of this amylose-lipid complex effectively inhibits enzymatic access to starch chains, thus reducing digestibility and enhancing RS content.

Compared to R0, all samples have a sharp and narrow peak at 1,746 cm^{-1}, which is associated with –C=O stretching vibration [34]. It is thought that lauric acid is the main contributor to the appearance of ester

groups. Peaks at 1,158 and 1,159 cm^{-1} show asymmetrically stretching vibration of the C–O–C bond. This is due to the existence of ether groups found in triglycerides. The spectrum of R0 Buras showed an absorption band at 2,926 cm^{-1}, indicating C–H stretching [18]. The additional band of 1,654 cm^{-1} indicates an α-helical structure [35], which is only found in R0 as a non-lipid component. This implies that the α-helical linkages in R1, R2, R3 and R4 might be weakened or deformed by lipids.

Effect of cooling-reheating on RS content and starch digestibility

Buras based on the highest RS content and lowest starch digestibility were then used for cooling-reheating treatment. Cooling-reheating was conducted because it was suspected to increase RS content and decrease starch digestibility. Cooling-reheating was conducted for 0, 1 and 2 cycles on the selected Buras (R1) and cssontrol (R0). The RS content and enzymatic hydrolysis curves of Buras are shown in **Figures 5(a)** and **5(b)**, respectively.

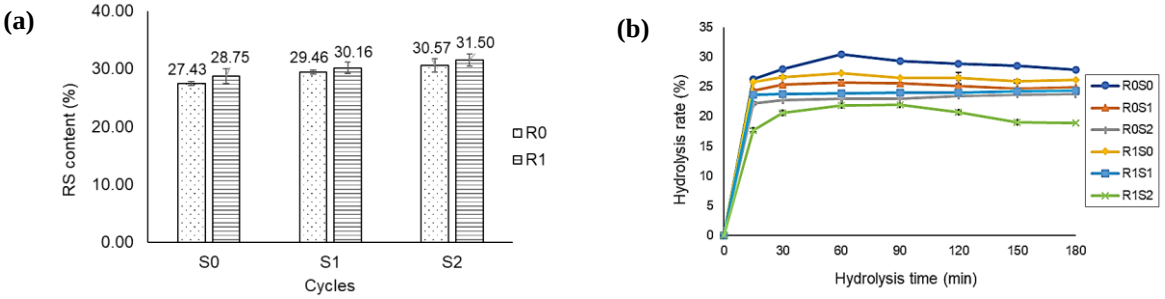

Figure 5 RS content (a); and enzymatic hydrolysis curve of Buras starch (b), where: R0 (no coconut milk added) and R1 (100 mL water: 100 mL coconut milk) after cooling-heating cycle treatment, where: S0 = freshly cooked Buras; S1 = 1× cooling-heating; and S2 = 2× cooling-heating.

Buras R1S2 had the highest RS content (31.50 %) (**Figure 5(a)**) and the lowest starch digestibility (18.94 % at 180 min) (**Figure 5(b)**). This proves that cooling-reheating for 2 cycles can increase the RS content, although it is not significant. The increase of RS through repeated cooling-heating refers to the phenomenon of retrogradation. Retrogradation has the principle of re-formation or recrystallization especially in the amylose structure. This recrystallization occurs when the amylose chain forms a double helix tightly bound by hydrogen bonds [21]. According to Wiruch *et al.* [36], gelatinization due to heating causes amylopectin to break down to form a single helix bond of amylose, then cooling causes amylose to form a double helix. As a result, RS content increases and decreases digestibility. Retrogradation is caused spontaneously when rice is cooled at 4 °C [18], thus reducing digestibility as shown by the increase in RS content (**Figure 5(a)**). Similar research was also reported by Ratnaningsih *et al.* [37] regarding the impact of the temperature, autoclaving followed by the cooling process caused changes to the microstructure and increased the RS content of cowpea starch. The amylose-lipid complex forms a V-type crystalline structure that is thought to trigger inhibition of α–amylase hydrolysis. According to Anugrahati *et al.* [6], the crystalline starch structure is more stable than the amylose-lipid complex.

Conclusions

Coconut milk ratio and cooling-reheating cycle factors increased resistant starch in Buras. Buras R1 or Buras with a coconut milk ratio of 1:1 (100 mL water: 100 mL coconut milk) resulted in the highest RS content (30.55 %) and the lowest starch digestibility. Cooling-reheating with 2 cycles increased the RS content of the R1 Buras sample by 9.57 % and had the lowest digestibility at 180 min (18.94 %). Therefore, the best-treated sample was R1 with 2 cooling-reheating cycles (R1S2). XRD and FTIR analysis confirmed the presence of an amylose-lipid complex. XRD analysis showed diffraction peaks of 17, 20 and 21 ° (2θ) in all Buras samples with coconut milk added. These peaks indicate the amylose-lipid complex and the semi-crystalline phase of the samples. Although in the semi-crystalline

phase, this corroborates that the Buras sample has a high RS content. The existence of an amylose-lipid complex in FTIR analysis is indicated by the bands appearing at 2,925 and 2,585 cm^{-1} due to the stretching vibration of the –C–H. SEM analysis shows that the surface of Buras starch granules appears smooth and shiny. The granule surface tends to be altered due to gelatinization and the presence of fat.

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