Canrea Journal: Food Technology, Nutritions, and Culinary, 2025; 8 (2): 343-359 OPEN ACCESS

e-ISSN : 2621-9468



*Type of the Paper (Article)*

# Effect of coconut milk-rice ratios and cooling-reheating cycles in amylose-lipid complex of *buras*: Indonesian traditional rice cake

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## Abstract

Resistant starch (RS), particularly RS5, has attracted considerable scientific interest due to its potential health-promoting effects, including reduced risk of degenerative diseases. However, research on RS5 in commonly consumed food products, like *Buras*, a traditional Indonesian dish, remains limited. *Buras* is made by cooking rice with coconut milk, which contains starch and lipids that can interact to form amylose-lipid complexes. The present study evaluates the effect of varying rice-to-coconut milk ratios and multiple cooling-reheating cycles on the formation of RS5 in *Buras*. The experimental method involved preparing *Buras* with four different rice-to-coconut milk ratios: P1 (1:1), P2 (1:2), P3 (1:3), and P4 (1:4). After cooking, *Buras* were subjected to multiple cooling-heating cycles, where they were cooled at 4°C for 6 hours and reheated in a steamer for 10 minutes. Control samples (S0) underwent no cooling-reheating cycles. Several parameters were measured, including amylose content, RS, starch digestibility, physicochemical characteristics, and complexing index (CI). Results indicated that the P2 ratio (1:2) produced the highest RS (7%) and significantly reduced starch hydrolysis. The amylose-lipid complex in *Buras* was confirmed through XRD peaks at 13°, 17°, and 20°, as well as FTIR ester group signals. Additionally, multiple cooling-reheating cycles further increased RS content while reducing starch digestibility. The findings suggest that the rice-to-coconut milk ratio and post-cooking processing are crucial for optimizing RS5 formation in *Buras*, offering potential health benefits and improving its nutritional profile. This research contributes to modernizing traditional recipes while maintaining their cultural significance.

**Article History**
Received November 30, 2024
Accepted November 26, 2025
Published December 9, 2025

**Keywords**
RS5, Amylose-Lipid, *Buras*, Starch Digestibility, Cooling-Reheating Cycles.

## 1. Introduction

Resistant starch (RS) exhibits physiological behavior similar to that of dietary fiber, as it is not broken down by digestive enzymes in the small intestine. RS is currently an interesting research trend because it has many health benefits (1), especially in reducing the risk of degenerative diseases that are currently a global threat. RS has 5 types, namely RS1 physically resistant (trapped within food structure), RS2 naturally resistant (raw, granular form), RS3 physically modified (cooked and cooled starch), RS4 chemically modified (cross-linking or esterification), and RS5, which are amylose-lipid complexes that are complex during gelatinization and retrogradation (2). RS5 is a new type of RS and is currently being widely developed. So far, RS5 has only been studied in flour-based models with the addition of lipids.

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ISSN 2621-9468 online, DOI 10.20956/canrea.v8i2.1486

There is limited research on the presence and characterization of RS5 in food products commonly consumed by the general population.

One of the starch-lipid-based food products similar to the concept of RS5 is *Buras*, a traditional Indonesian culinary specialty of the Bugis-Makassar tribe in South Sulawesi province. *Buras* are processed in a unique way, with banana leaves used to wrap them during cooking. Rice, as the raw material for *Buras*, contains a total starch of 70-80% of the dry weight, and amylose consists of around 15-25% of the total starch (3). In addition, coconut milk as the raw material for *Buras*, contains saturated fat, which makes up most of the fat content in coconut milk, around 80-90% of the total fat (4). Adding coconut milk to rice during the cooking process might form an amylose-lipid complex called RS5. Amylose can form a complex more easily than amylopectin because the linear and helical structure creates a more suitable environment for binding lipid molecules. Conversely, the branched and large structure of amylopectin makes it less efficient in binding lipids (5). Factors affecting the formation of amylose-lipid complexes include the ratio of starch-lipid-based raw materials, amylose content, lipid content, and post-cooking product processing methods.

One of the factors that can affect the development of amylose-lipid complexes throughout the preliminary preparation of *Buras* is the ratio between the addition of rice and coconut milk. The ratio of rice and coconut milk affects the availability of amylose (from rice) to interact with lipids in coconut milk. The appropriate formulation is essential in enabling the development of amylose-lipid complexes. An Imbalance, either more or less of one component, can disrupt this complex. Therefore, the ideal ratio ensures that amylose is able to form a helical structure that traps lipids well so that optimal RS5 is formed.

Besides the ratio between rice and coconut milk, physical modification of post-cooking processing can potentially increase the amylose-lipid complex. The mechanism occurs when amylose-lipid is stored at a cold temperature. It will retrograde the complex and increase crystallinity, making it more digestive enzyme-resistant, and RS increases (6). Then, if reheating occurs, amylose leaches from starch granules and forms a helix with more stable lipids. Therefore, the physical modification factor after cooking with several cycle variations must be studied further to evaluate the enhancement of resistant starch levels alongside the reduction in starch hydrolysis.

Accordingly, the present research aims to evaluate the impact of different coconut milk-rice ratios and multiple cooling-reheating cycles on the amylose-lipid complex formation in *Buras*. Applying repeated cooling-reheating cycles is expected to facilitate a synergistic increase in the formation of RS type 5 (RS5). Each cycle enhances the interaction between amylose and lipid components, leading to more stable and enzyme-resistant complexes. By understanding how these variables affect the crystallinity and RS content, this research will provide insights into the preparation of *Buras* to improve its nutritional profile, particularly by increasing its RS5 content. This could offer potential health benefits and help modernize traditional recipes while maintaining their cultural significance. This research sets the stage for a study exploring the role of ingredients and processing conditions in modifying starch properties in traditional Indonesian food. The study will address both the biochemical mechanisms and potential health benefits of RS.

## 2. Materials and Methods

### 2.1. Materials

The main materials of *Buras* are rice, the varieties Setra Ramos. The Setra Ramos rice variety used in this research exhibits an amylose content of approximately 25%, determined using the iodine-binding colorimetric method (7) and commercial coconut milk (Kara, Indonesia) obtained from supermarkets in Sleman, Yogyakarta. The additional materials are bay leaf and salt. The main chemicals used for analysis include iodine ($I_2$ and KI), buffer solution KCl-HCl pH 1.5, buffer tris-maleate solution 0,1 M pH 6.9, buffer sodium acetate solution 0.4 M pH 4.75, α-amylase enzyme (Sigma A3176, Sigma-Aldrich Inc., USA), pepsin enzyme (Sigma P7000, Sigma-Aldrich Inc., USA), and GOD-FS (Ref 1 2500 99 83 021, DiaSys Diagnostic Systems GmbH, Germany).

### 2.2. Preparation of Various Ratios of Rice and Coconut Milk

*Buras* were made with 4 different ratios (rice : coconut milk). Rice was measured using an analytical balance, and coconut milk was measured with a measuring cup. These ratios (b/v) are: P1 = 1:1 (100 g rice: 100 mL commercial coconut milk), P2 = 1:2 (100 g rice: 200 mL commercial coconut milk), P3 = 1:3 (100 g rice: 300 mL commercial coconut milk), and P4 = 1:4 (100 g rice: 400 mL commercial coconut milk). *Buras* is then cooked using each of these different ratios.

### 2.3. Buras Making Procedure

Approximately 150 g of rice was rinsed with tap water and then mixed with coconut milk at different ratios (P1–P4). The procedure for making *Buras* is shown in Figure 1.


Figure 1. Procedures for making *Buras*.

Based on Figure 1, the coconut milk used in each formulation was prepared from the raw material by diluting commercial coconut milk with water at a 1:1 (v/v) ratio. The rice and

coconut milk mixture was then cooked in a rice cooker (Cosmos CRJ-6123, Indonesia) until it automatically switched off (approximately 20 min). After cooking, the rice was allowed to cool at ambient temperature for 10 min. Two tablespoons of cooked rice were taken, then wrapped in banana leaves into pre-gelatinized *Buras* sized 8 cm x 5 cm x 2 cm in a rectangular shape. All samples were wrapped in the same type of fresh banana leaf before the cooling cycle, and the same packaging material was used consistently across all treatments to ensure uniform heat transfer and moisture retention conditions. These pre-gelatinized *Buras* were tied using plastic rope and then boiled for 60 min in boiling water using a gas stove (Modena FC 3955, Modena Technology Ltd., Italy). After cooking and gelatinization, the *Buras* were cooled to room temperature for 1 h.

### *2.4. Multiple Cooling-Reheating Cycles for Buras*

The cooling-reheating process for *Buras* involves storing it at 4 ℃, then heating it for 10 min in a steamer every 6 h in each cycle. At the same time, the treatment for *Buras* without a cycle (S0) did not involve storage at 4 ℃ and repeated heating. All samples were reheated by steaming at 100 °C for 10 min using the same steamer and batch size to maintain consistent heat exposure among treatments.

### *2.5. Sample Preparation*

The *Buras* preparation is ground into a powder, placed in the freezer for 24 h, and then transferred to a cup with a small hole. The frozen *Buras* were then put into a freeze dryer for 24 h. After that, the *Buras* were refined and filtered with a 100-mesh sieve.

### *2.6. Determination of Amylose Content*

Amylose content was determined using the analytical procedure reported by Juliano (7). The sample was measured at 0.1 g and put into a 100 mL volumetric flask. 1 mL of 95% ethanol and 9 mL of 1 N NaOH were added to the sample. The mixture was heated with boiling water for 10 min and cooled. The sample was diluted with distilled water until the 100 mL tare mark. The sample solution was diluted to 5 mL, then placed in a 100 mL volumetric flask, and 1 mL of 1 N acetic acid and 2 mL of 0.2% iodine were added. The sample solution was diluted up to the mark. The sample solution was incubated for 20 min, and then the absorbance was measured with a wavelength of 625 nm.

### *2.7. Determination of Complexing Index (CI)*

Analysis of the complexing index according to Liu *et al*. (8). The sample was weighed to 0.2 g, and 10 mL of deionized water was placed in centrifuged tubes. The aliquot was then heated for 30 min. The aliquot was then centrifuged for 10 min at 4000 × g. The supernatant was 50 µL, and 2 mL of iodine (1.3% $I_2$ and 2% KI in deionized water) was added. Then, the sample was tested with a UV-vis spectrophotometer at a wavelength of 690 nm.

$$\text{CI } (\%) \frac{(\text{Absorbance of control-Absorbance of sample})}{\text{Absorbance of Control}} \text{ x } 100 \quad (1)$$

### *2.8. Determination of Resistant Starch Content*

The RS was determined using the enzymic method of Goni *et al*. (9). A 25 mg sample powder was placed into a 15 mL falcon tube with 2.5 mL of KCl-HCl buffer solution, then vortexed. The sample was added with 50 µl of pepsin solution, then vortexed, incubated at

40 ℃ for 60 min, and cooled. Next, 2.25 mL of 0.1 M tris-maleate buffer pH 6.9, and 250 µL of α-amylase solution were added, and the mixture was incubated at 37 ℃ for 16 h. The sample was centrifuged for 15 min at 3000 rpm, and the supernatant was discarded. The remaining residue was washed with 10 mL of distilled water, then centrifuged again, and the supernatant was discarded. The residue formed was added with 3 mL of distilled water and 0.75 mL of 4 M KOH solution and incubated for 30 min at 37 ℃ with constant vibration. The sample solution was mixed with 1.375 mL of 2 M HCl, 0.75 mL of sodium acetate buffer, and 20 µL of the amyloglucosidase enzyme from *Aspergillus niger* (Sigma A7095, Sigma-Aldrich Inc., USA). Then it was incubated in an incubator shaker at 60 ℃ for 45 min. The sample was centrifuged twice for 15 min at 3000 rpm, and the supernatant was transferred to a 25 mL measuring flask and diluted. The test was carried out by adding 0.5 mL of the sample solution to a test tube and incubating 1 mL of the GOD solution for 30 min at 37 ℃. Then the absorbance was measured at a wavelength of 510 nm using a UV-Vis spectrophotometer.

### *2.9. In Vitro Digestibility*

In vitro digestibility procedure following Goni *et al*. (10). The sample weighed as much as 50 mg, then incubated with 10 mL of KCl-HCl buffer pH 1.5, and homogenized for 2 min. Then 0.2 mL of pepsin (1 g in 10 mL of KCl-HCl buffer) was added and incubated for 1 h at 40 ℃ in a water bath shaker. The sample solution was diluted to 25 mL with tris-maleate buffer pH 6.9. Then, 5 mL of α-amylase enzyme (2.6 IU) was added and incubated at 37 ℃ in a water bath shaker. 1 mL aliquot was taken from each sample every 30 min from 0 to 3 h. The aliquot sample was placed in a test tube heated at 100 ℃ for 5 min to inactivate the enzyme and synchronize until the end of the incubation time. Then, 3 mL of 0.4 M Sodium Acetate buffer pH 4.75 and 60 µL were added to each aliquot and incubated for 45 min at 60 ℃ on a water bath shaker. Then, the sample was diluted from 10 to 100 mL with distilled water. The solution sample was taken 0.5 mL, and 1 mL of GOD was added. Glucose was converted into starch by multiplying by 0.9. The starch digestion rate was expressed as the percentage of Total Starch (TS) hydrolyzed at different times (30, 60, 90, 120, 150, and 180 min).

### *2.10. Scanning Electron Microscopy (SEM)*

The morphological characteristics of Buras granules were examined using a Scanning Electron Microscope (JSM-6510 Series, Japan). Samples were affixed onto metal stubs with black conductive tape before imaging. To enhance conductivity, each specimen was sputter-coated with a thin layer of gold under vacuum. The granular surface features of Buras were then visualized at a magnification of 1,000× using an accelerating voltage of 10 kV.

### *2.11. X-Ray Diffraction*

X-ray diffraction (Bruker D8 Advance Eco, Germany) was operated at 25 ℃ (room temperature), the anode was Cu, generator kV was 40 kV, generator mA was 25 mA, and the wavelength for display was 1.5406 Å. The diffraction spectrum was recorded over 5–35$^{o}$ (2θ) for 76.80 s, with a step size of 0.020$^{o}$ and a time per step of 0.40 s.

### *2.12. 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). The samples before analysis were mixed with KBr powder (1:100, w/w) in an agate mortar. Samples were then pressed in a nut between

two tightened bolts, resulting in transparent pellets for measurement. The spectra were obtained in the 4000–400 $cm^{-1}$ range at 4 $cm^{-1}$ resolution.

### *2.13. Statistical Analysis*

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

## 3. Results and Discussion

### *3.1. Complexing Index (CI) of Amylose-Lipid*

The Complexing Index (CI) quantifies the extent of amylose-lipid complex (ALC) formation within a substance. This index provides insights into how much amylose interacts with lipids to form stable complexes, which is particularly relevant in food science for understanding the development of RS5 and digestibility. The CI reflects the degree of molecular interactions between amylose and lipid components, which can influence the physicochemical characteristics of starch, including crystalline structure and digestibility. The effect of the ratio of rice and coconut milk on amylose-lipid complex formation in *Buras* are presented in Table 1.

Table 1. Effect of the ratio of rice and coconut milk on amylose-lipid complex formation in *Buras*

| Treatment | Complexing Index |
|---|---|
| P1 (1:1) | 32.69 ± 0.75[a] |
| P2 (1:2) | 37.03 ± 0.81[a] |
| P3 (1:3) | 27.51 ± 0.61[b] |
| P4 (1:4) | 22.70 ± 0.12[b] |

*Data are expressed as mean ± standard deviation. Different letter(s) in the same column indicate significant differences according to one-way ANOVA (p<0.05)*

Based on Table 1, P2 (1:2) shows the highest CI, while P4 (1:4 ratio) exhibits a lower CI. P2 (1:2 ratio) has a balanced proportion of rice (amylose) and coconut milk (lipid), which facilitates the formation of a more stable amylose-lipid complex. The optimal amount of lipids in P2 allows amylose to interact more efficiently with the lipids, forming well-organized helical structures or amylose-lipid complexes. Amylose-lipid complexes are more stable when the ratio is such that enough amylose is present to form a helix that can trap lipid molecules (1). In P2, the available lipid content is sufficient to bind amylose into a tight and stable complex, increasing its resistance to enzymatic digestion and making the starch more crystalline (retrograded structure), contributing to a higher CI.

In contrast, P4 (1:4 ratio) contains a higher amount of coconut milk (lipid) relative to rice (amylose), which can lead to an excess of lipids. When the lipid content exceeds the amylose content, the complex may not form optimally. Excess lipids can disrupt the formation of stable amylose-lipid complexes, as too much lipid can interfere with the ability of amylose to form a well-ordered helix structure (11). Instead of creating a stable, crystalline amylose-

lipid complex, an excess of lipids can result in a less organized complex, leading to a lower CI (12). In P4, the higher lipid concentration may also cause the amylose to remain more amorphous, meaning that the amylose-lipid complex lacks the dense, crystalline structure that enhances RS formation (13). Excess lipid can prevent amylose from retrograding properly, thereby lowering CI. However, due to excess lipids, the retrogradation process in P4 may be less efficient, resulting in fewer crystalline amylose-lipid complexes and a lower CI.

### *3.2. The Effect of Ratio Composition on Amylose and RS5 Content*

The ratio of coconut milk to rice is crucial in determining the nutritional characteristics of *Buras*. Coconut milk is rich in fats, which can interact with starch molecules in rice, particularly amylose, to form amylose-lipid complexes. These interactions influence the formation of resistant starch (RS5), a type of starch that resists digestion in the small intestine, contributing to a lower glycemic index and beneficial effects on gut health. The data presented in Table 2 shows the influence of different coconut milk-to-rice ratios on amylose content and RS5 formation in *Buras*.

Table 2. Effect of ratio composition on amylose and RS5 content of *Buras*

| Treatment | Amylose (%) | RS Content (%) |
|---|---|---|
| P1 (1:1) | 27.32 ± 0.71$^{a}$ | 16.6 ± 1.15$^{a}$ |
| P2 (1:2) | 28.85 ± 0.69$^{a}$ | 17.07 ± 0.75$^{a}$ |
| P3 (1:3) | 25.75 ± 0.44$^{b}$ | 15.42 ± 0.41$^{b}$ |
| P4 (1:4) | 23.21 ± 0.58$^{c}$ | 14.80 ± 0.29$^{b}$ |

*Data are expressed as mean ± standard deviation. Different letter(s) in the same column indicate significant differences according to one-way ANOVA ($p<0.05$)*

RS content shows an upward trend in relation to amylose levels, as seen in Table 2. The treatment with the highest resistance content is the 1:2 ratio of rice to coconut milk (P2). A 1:2 ratio allows more coconut milk to reach the lipids needed to interact with the amylose in rice. As a result, the amylose-lipid combination becomes more stable. The optimal development of this complex, which in turn maximizes the RS concentration, is ensured by the balance between lipid and starch (amylose). Lower RS concentration results from treatments with a higher or lower ratio of rice to coconut milk (like P1 or P3) because there is insufficient lipid for efficient complex formation or because the starch-lipid ratio is less optimal. Amylose, a linear polymer of glucose, has the ability to form complexes with lipids, particularly under conditions of heat and moisture. These amylose-lipid complexes alter the starch's digestibility by increasing its resistance to enzymatic breakdown, thereby enhancing the formation of RS (14). The interaction between amylose and lipids reduces the enzymatic accessibility of amylose, thereby increasing RS content. The lipid molecules hinder the enzymatic action on amylose, contributing to RS formation (15). In treatment P2, the ratio of rice to coconut milk (1:2) provided an optimal amount of lipids, facilitating the formation of amylose-lipid complexes without excess lipid interference. This balanced interaction led to higher levels of both amylose and RS in P2 compared to the other treatments.

At the molecular level, the amylose-lipid complex (RS5) exhibits greater resistance to α-amylase hydrolysis than retrograded starch (RS3) because of its distinct structural configuration. In RS5, lipid molecules are embedded within amylose helices, forming V-type

crystalline structures with hydrophobic interiors that shield glycosidic bonds from enzymatic attack. This hydrophobic barrier limits α-amylase binding and reduces the enzyme's accessibility to the starch chains. Moreover, the tight helical conformation and crystalline packing of RS5 enhance its structural rigidity and decrease hydration, further impeding enzyme penetration. In contrast, RS3 primarily consists of hydrogen-bonded amylose or amylopectin double helices that are more accessible to α-amylase. Consequently, RS5 exhibits greater resistance to enzymatic hydrolysis, resulting in increased RS content in P2.

In contrast, increasing the coconut milk ratio beyond this level, as in P4, did not enhance RS formation. Excess lipid addition increases the viscosity of the starch-lipid matrix, restricting amylose mobility and hindering the proper alignment required for complex formation. The high lipid concentration may also result in incomplete starch gelatinization due to the coating effect of fat, which limits water absorption and starch swelling. Furthermore, excessive saturated fats in coconut milk can create physical barriers or promote phase separation during cooling, thereby reducing the effective contact between amylose and lipid molecules necessary for stable complexation. The higher coconut milk ratio may also have increased moisture and diluted amylose within the starch matrix, while non-lipid components such as proteins could have competed with amylose for lipid interaction, further limiting RS5 formation.

### *3.3. The Effect of Ratio Composition on Starch Digestibility*

The digestibility of starch refers to the extent to which digestive enzymes can break down starch into simpler sugars. When amylose in starch combines with lipids, it forms amylose-lipid complexes less susceptible to enzymatic digestion. This results in a higher proportion of RS, which is not digested in the small intestine but is fermented in the large intestine, promoting gut health and altering the glycemic response. An ideal ratio will promote the formation of RS without compromising other aspects of the food product. This balance is necessary to improve starch resistance to digestion while maintaining product quality. The effect of ratio composition on Strach digestibility is shown in Figure 2.

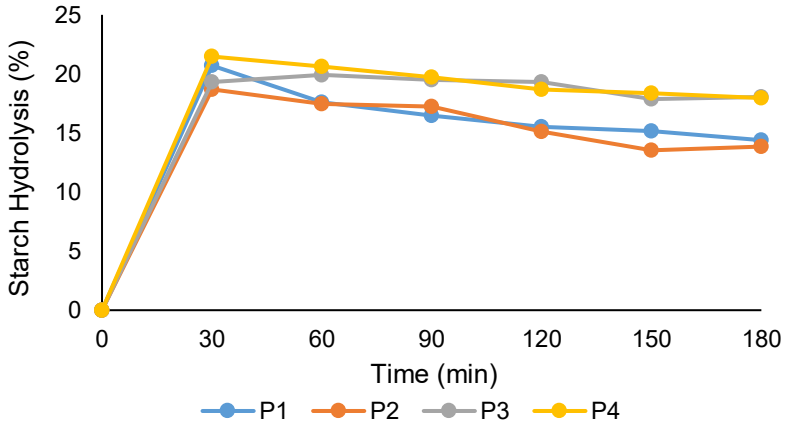


Figure 2. Hydrolysis of starch from *Buras* with varying ratios of rice and coconut milk, there are: P1 = 1:1; P2 = 1:2; P3 = 1:3; and P4 = 1:4.

According to Figure 2, the P2 (1:2) ratio likely results in the highest level of amylose-lipid complex formation among the treatments. In this case, the increased amount of coconut milk provides more lipids, which interact with amylose in the rice, significantly reducing starch

digestibility and increasing RS content. The higher lipids facilitate the formation of stable amylose-lipid complexes, making the starch more resistant to enzymatic breakdown (16). This results in higher RS content than other treatments, as observed in the previous analysis, where P2 exhibited the highest RS and amylose content. In P2, with a 1:2 rice-to-coconut milk ratio, there is a more balanced interaction between the rice starch (amylose) and coconut milk lipids. At this ratio, the amount of lipids is sufficient to form amylose-lipid complexes without overwhelming the starch. This allows for a more optimal formation of RS. In this case, the complex is stable, reducing the starch's digestibility and increasing the RS content (17). The P2 ratio likely results in the ideal environment for amylose-lipid complex formation. The amount of lipids present is enough to decrease starch digestibility by forming RS. However, it is not so excessive that it disrupts the starch's structure or prevents the complex from forming effectively (18). This leads to higher RS content in P2 compared to P4 (1:4).

The low RS content in P4 is likely due to the fact that too much lipid is available relative to the rice starch. In the ideal scenario, starch molecules (especially amylose) form complexes with lipids under specific conditions (heat, moisture). However, in P4 (1:4), the overwhelming lipid content likely prevents proper amylose-lipid interaction, essential for forming RS. The excess lipids may also lead to free lipids that do not effectively interact with amylose, reducing the overall RS formation. Excessive coconut milk (lipids) of P4 might cause starch granules to absorb more liquid and become more soluble. This increased solubility of starch could make it easier for digestive enzymes (like amylase) to break down the starch into simpler sugars. As a result, P4 has the lowest RS because the starch is more prone to hydrolysis and digestion, making it less resistant to breakdown in the small intestine.

At the molecular level, the amylose-lipid complex (RS5) demonstrates greater resistance to α-amylase hydrolysis than retrograded starch (RS3) because of its hydrophobic and compact crystalline structure. The embedded lipid molecules form a hydrophobic barrier within the amylose helix, limiting enzyme binding and substrate accessibility. In contrast, RS3 is stabilized primarily by hydrogen bonding, making it less compact and more susceptible to enzymatic attack. The hydrophobic shielding and rigid crystalline arrangement of RS5 therefore make it more effective in inhibiting α-amylase activity, resulting in lower starch digestibility and higher RS content in the P2 treatment.

### *3.4. Morphology of Buras*

The grounded *Buras* samples were analyzed for granule morphology through a Scanning Electron Microscope (SEM). This analysis showed differences in the granule morphology of *Buras* made with different ratios of rice and coconut milk. SEM images of the *Buras* are shown in Figure 3.

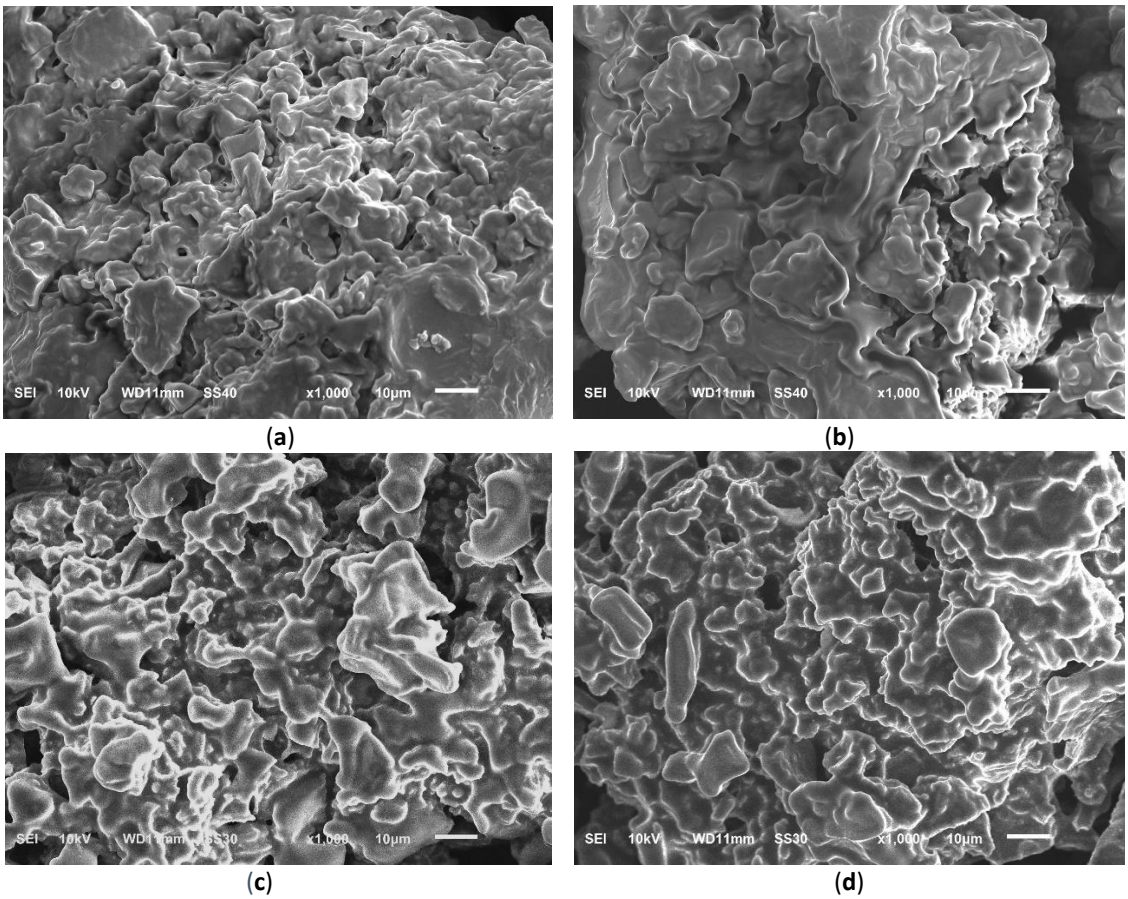


Figure 3. SEM of the *Buras* surface with varying ratios of rice and coconut milk, there are: (a) P1 = 1:1; (b) P2 = 1:2; (c) P3 = 1:3; and (d) P4 = 1:4.

The granule surface of *Buras* with all treatments in Figure 3 showed an aggregated shape. This is thought to be a contribution from the coconut milk, especially the fat. The surface appearance of the *Buras* appears solid, and the original shape of the granules can still be seen, although it has been slightly destroyed (a, b). The *Buras* structure (c, d) formed cavity gaps and looked irregular due to damage. As stated by Wang *et al*. (19) that mixtures with increasing concentrations of fatty acids more clearly damage starch granules. The surface also appeared luminous due to the fat covering the granule surface. This is due to the increasing amount of coconut milk added.

The cooking method that caused gelatinization also contributed to the change in granule shape. Steaming and reheating processes that involve heat cause swelling of the *Buras* granules. This was consistent with Park *et al*. (20) that the granule expands, and its structure is disrupted when the heating temperature rises during gelatinization. Cooling-reheating, applied over several cycles, further regularized the *Buras* surface.

### *3.5. Starch Crystallinity*

The crystallinity of *Buras* with various treatments was analyzed using X-ray diffraction (XRD). This technique is based on X-rays being emitted onto the crystal structure of the material and diffracted at an angle of 2θ. The resulting diffraction pattern interprets the crystalline characteristics of the sample, such as the degree of crystallinity, crystalline phase, or material phase (crystalline or amorphous). The presence of amylose-lipid complexes or V-

type crystal characteristics is indicated by a minor peak at least 7° and major peaks at 13° and 20° (21). Starch crystallinity is shown in Figure 4.

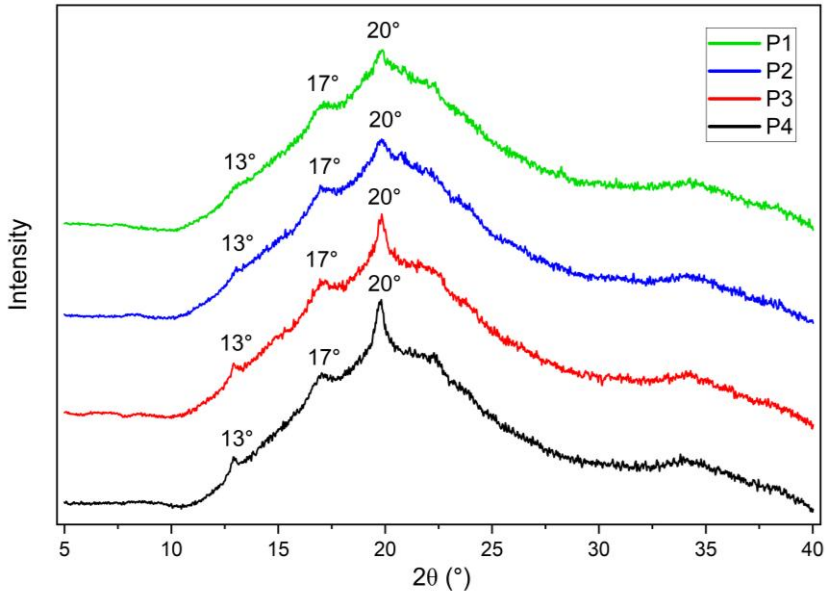


Figure 4. Crystallinity from *Buras* with varying ratios of rice and coconut milk, there are: P1 = 1:1; P2 = 1:2; P3 = 1:3; and P4 = 1:4.

Figure 4 shows that the *Buras* with all treatments have the same peaks at 13°, 17°, and 20°. Samples P1, P2, P3, and P4 have amylose-lipid complexes, indicated by the 13° and 20° peaks. Meanwhile, the 17° peak comes from starch recrystallization (22). This shows that all samples have a v-type crystal structure, but are less perfect because they only have peaks at 13° and 20°. Although the peaks are the same, their intensities vary depending on the peak shape. Sharper and narrower peaks indicate higher crystal regularity, while wide or blunt peaks indicate a less regular crystal structure. P1 and P2 have blunt and wide peaks, possibly due to the lower amount of coconut milk added. The peaks of P3 and P4 are higher than P1 and P2, this is thought to be due to the more coconut milk added, causing more fatty acids to bind with amylose to form lipid-amylose complexes. As all samples only have 2 of the 3 peaks of the type-v crystal structure (13° and 20°), and the peaks are generally blunt, so the phase of all *Buras* starches tended to be semi-crystalline.

P2 (1:2) treatment likely contains a moderate amount of lipids that interact effectively with the starch, forming amylose-lipid complexes while preserving a good portion of the starch's crystalline structure. In the XRD pattern, you might observe the characteristic crystalline peaks of amylose and amylopectin, though the peak intensities may be slightly reduced compared to the untreated starch. This suggests that the starch is still relatively ordered but has undergone some modification due to lipid interaction. In contrast, P4 has a much higher lipid content, which might lead to a more disordered starch structure. The XRD pattern would likely show a significant reduction in the intensity of crystalline peaks and increased broadening of the peaks, indicating a more amorphous state. This suggests that the excessive lipids interfere with the starch's ability to maintain its crystalline structure, leading to a lower degree of crystallinity and potentially a higher starch digestibility. The reduced level

of crystalline order in P4 can be associated with its lower RS content, as the starch is more easily digested when its structure is less ordered.

### 3.6. Functional Groups

The functional groups found in *Buras* can be determined by FTIR analysis. This information was especially important about functional groups detected due to the interaction of coconut milk fat with rice starch in various ratios. IR spectra of *Buras* with different variations in the ratio of rice and coconut milk (Figure 5) show that all samples have uniform absorption bands. The formation of the starch-fatty acid complex triggered the appearance of peaks in the FT-IR spectrum, namely 2854 and 1746 $cm^{-1}$. The added coconut milk was thought to form amylose lipid complexes between rice amylose and coconut milk fatty acids. The absorption bands at 2925 and 2854 $cm^{-1}$ indicate asymmetric stretching vibrations of –CH3 and –CH2, while 1746 $cm^{-1}$ is associated with stretching vibrations of –C=O in coconut milk fat. The 1158 $cm^{-1}$ band is related to C–O–C stretching vibrations derived from oxygen bonds in the anhydrous-glucose ring of starch (23).

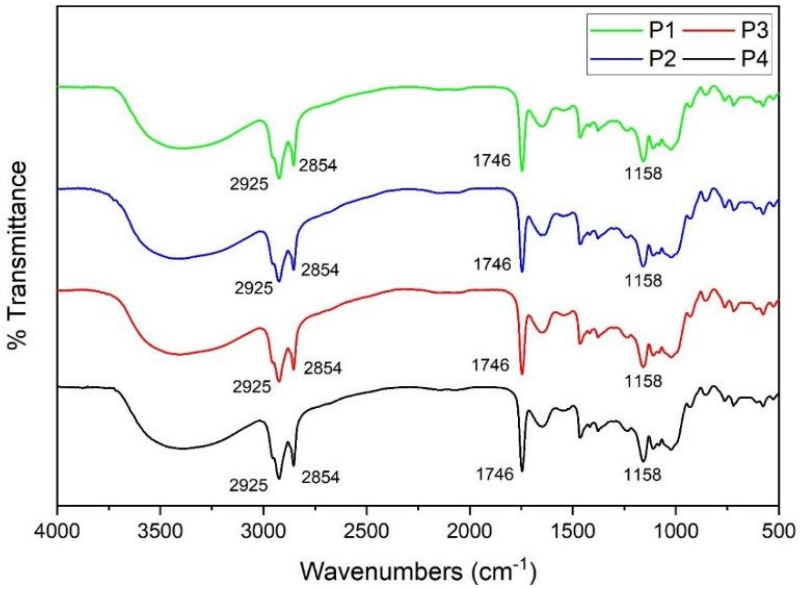


Figure 5. IR spectrum from *Buras* with varying ratios of rice and coconut milk, there are: P1 = 1:1; P2 = 1:2; P3 = 1:3; and P4 = 1:4.

The band intensities for each sample were relatively similar (Figure 5). This indicates that the amount of coconut milk added does not affect the functional composition or chemical structure of the analyzed *Buras* samples, as evidenced by the absence of significant differences in the intensities of the absorption bands at 2925, 2854, 1746, and 1158 $cm^{-1}$ across samples with different coconut milk ratios. These absorption bands are most likely related to vibrations of major functionalities in the base material, such as fatty acids (at 2925 and 2854 $cm^{-1}$) and esters (at 1746 $cm^{-1}$), which were not significantly affected by variations in coconut milk concentration in the formulation. However, the steaming process method used is thought to affect the absorption bands that will be detected. This is because steaming can cause amylopectin not to maintain its double-helical structure, resulting in weak hydrogen bonds inside or outside the molecule (24).

### 3.7. The Effect of Cooling-Reheating Cycles on RS5 Content and In Vitro Digestibility

The subsequent experimental modification involves subjecting P2 to cooling-reheating cycles to further study the structural changes in the starch and their effects on the RS content and digestibility. P2 exhibited the highest RS content, indicating that the molecular association occurring between amylose and lipid components under the 1:2 rice to coconut milk ratio produced the most stable amylose-lipid complexes. The effect of cooling-reheating cycles on RS5 content and in vitro digestibility is presented in Figure 6.

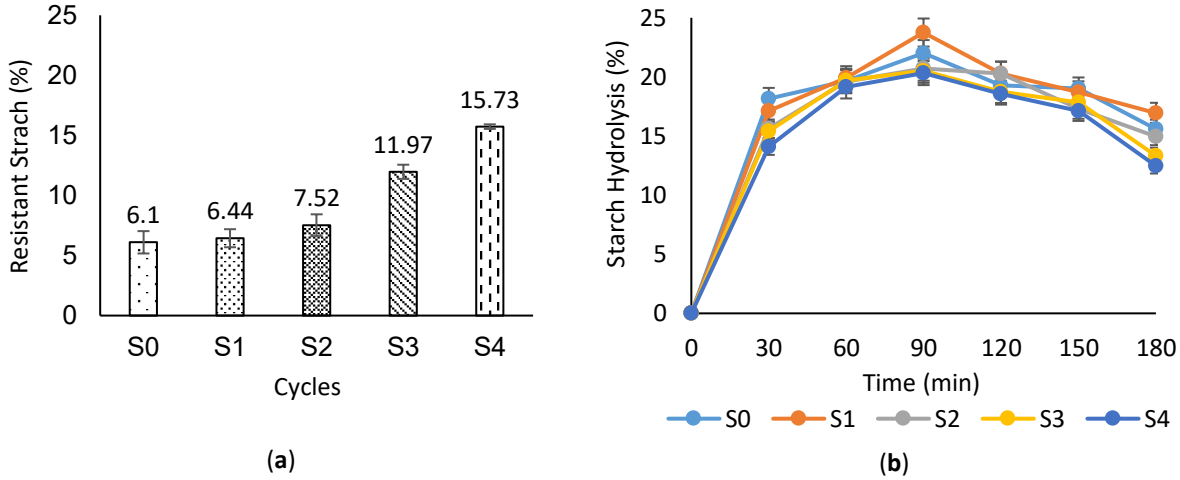


Figure 6. The Effect of Cooling-Reheating Cycles on RS5 Content and In Vitro Digestibility (S0 = No Cycle; S1 = 1 Cycle; S2 = 2 Cycles; S3 = 3 Cycles; S4 = 4 Cycles).

Cooling at 4 °C for 6 h promotes retrogradation of the starch. Retrogradation refers to the process of starch molecules, especially amylose, reassociating after gelatinization, resulting in the development of structures with a higher degree of crystallinity. The cooling period will likely enhance the RS content by making the starch more resistant to enzymatic digestion. Reheating the starch allows for partial gelatinization of the starch, potentially making it more digestible. However, reheating may also cause the retrograded starch to become more accessible to digestive enzymes, depending on the temperature and time. This may affect the digestibility of the starch but can also induce changes in the formation of RS. Each cooling-reheating cycle will alter the crystallinity and amorphousness of the starch, influencing its RS content and digestibility.

The cooling stage promotes the interaction between amylose and lipid molecules, forming complexes that are less digestible than native starch. This process leads to an elevation in RS content as the starch molecules undergo retrogradation, making them less accessible to digestive enzymes. Amylose molecules reassociate and form tighter, crystalline structures (18). Such structural modifications result in RS that exhibits reduced vulnerability to enzymatic degradation. Therefore, RS content is expected to increase following the cooling period. The reheating process causes the retrograded starch to partially re-gelatinize, which may reduce its crystallinity and make it more susceptible to enzymatic attack. Depending on the extent of this gelatinization, some of the RS may become more digestible after reheating. Treatment of S4 (4 cycles) builds up more RS over time (Figure 6). Since each cycle allows the starch to retrograde further, the RS content increases with each subsequent cycle. The crystalline structure of starch becomes more stable and less digestible, even though reheating might partially gelatinize the starch and increase digestibility (25). The effect of multiple

cooling phases likely outweighs this, leading to greater RS and lower digestibility. The control (S0), which undergoes no cooling-reheating cycles, will likely show lower RS content and higher digestibility. Without the retrogradation effect of cooling, the starch remains more gelatinized after cooking. It is more easily digested by enzymes, leading to higher digestibility and lower RS formation.

## 4. Conclusions

The ratio of rice to coconut milk influenced the increase in RS content in *Buras*. This is indicated by the highest RS content of 17.07% obtained through P2 *Buras*, which is a 1:2 ratio (rice: commercial coconut milk). This number is also indicated by the highest amylose content of 28.85%, the complexing index of 37.03%, and the lowest starch digestibility at 180 min. The most effective number of cooling-reheating cycles to increase RS levels was 4 cycles (S4), which is 24 hours of the total time, with 6 hours per cycle. This proves that the best treatment is for *Buras* with P2S4 (rice and coconut milk ratio of 1:2 and 4 cycles of cooling-reheating). The presence of amylose-lipid complex was confirmed using XRD, FTIR, and SEM analysis instruments. XRD analysis showed the presence of 17° and 20° peaks at the 2θ angle. FTIR absorption bands at 2925 and 2854 $cm^{-1}$ indicated the presence of fatty acids, and at 1746 $cm^{-1}$ for ester groups. The main bands indicated starch-lipid complexes were at 2854 $cm^{-1}$ and 1746 $cm^{-1}$. The *Buras* morphology observed by SEM showed an irregular, fissured structure due to gelatinization. The surface of the granules appeared luminous because they were covered with fat as the amount of coconut milk added to the *Buras* increased.

## Acknowledgments

This research and publication were supported by the Directorate of Research, Gadjah Mada University.

## Author Contributions

Y.P. designed experiments and wrote the original draft; A.M.S designed experiments, investigated, validated, and wrote the paper; M.A.P. investigated, interpreted, analyzed data, wrote, and edited the paper.

## Funding

This research received external funding from the Directorate of Research, Gadjah Mada University.

## Institutional Review Board Statement

Not applicable.

## Data Availability Statement

The manuscript presents the available data.

## Conflicts of Interest

The authors declared no conflict of interest.

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