Indonesian Food Science and Technology Journal
IFSTJ: Vol 9 No 1, Dec 2025 (PP:89-97)
ISSN: 2615-367X, DOI. 10.22437/ifstj.v9i1.44626

INDONESIAN FOOD SCIENCE AND TECHNOLOGY JOURNAL (IFSTJ)

Journal homepage : online-journal.unja.ac.id/ifstj/issue/archive

Resistant Starch Type 5 in Buras as Indonesian Traditional Food: Influence of Amylose Content and Multiple Cooling-Reheating Cycles

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***Abstract*— High-resistant starch foods are gaining attention for their potential to lower the risk of diabetes, obesity, and degenerative diseases. One type of resistant starch (RS) is RS5, a starch-lipid complex. This study investigated the effects of amylose content and physical modifications on Buras, a traditional Indonesian food made from rice and coconut milk. The physicochemical characteristics were evaluated using three rice varieties (Setra Ramos, C4 Super, and Rojo Lele) and cooling-reheating cycles (Control, 1 cycle, 2 cycles). Setra Ramos exhibits the highest amylose and RS5 contents at 25.14% and 26.54%, respectively. High amylose content facilitates the formation of lipid-amylose helical structures, inhibiting starch digestibility by enzymes such as α-amylase. RS5 in Buras was identified by the presence of amylose-lipid complexes, as indicated by Scanning Electron Microscopy (SEM) for visualization of granule structure, X-ray Diffraction (XRD) for crystallinity, and Fourier-transform infrared spectroscopy (FTIR) for functional groups. The best treatment was obtained at 1 cycle of cooling-reheating (S1), which increased RS by 9% and reduced starch hydrolysis by 11%. This study examined strategies to enhance RS5 content and reduce starch digestibility with physical modifications. The findings showed that cooling-reheating cycle treatment significantly ($p<0.05$) increased RS content in Buras. This increase correlated with increased starch retrogradation and the formation of amylose-lipid complexes, thereby reducing starch digestibility. Consequently, traditional food products become healthier and help control blood sugar levels. Therefore, simple thermal modification, such as cooling and reheating, improves the functional properties of starch-based traditional foods.**

***Keywords*— Buras; resistant starch; amylose-lipid complex; starch digestibility; retrogradation; cooling-reheating; traditional foods**

Manuscript received May 29, 2025; revised Dec 05, 2025; accepted Dec 12, 2025. Available online Dec 17, 2025

I. INTRODUCTION

The concept of resistant starch (RS) has revived interest in starch research, as it serves as a dietary fiber source with proven health benefits. RS refers to the fraction of starch that escapes digestion in the human gastrointestinal tract. Its relevance continues to grow as the global prevalence of type 2 diabetes and cardiovascular disease increases, underscoring the need for food products and processing methods that naturally enhance RS content. RS is classified by concentration: <1% as low, 1–2.5% as medium, and >15% as high RS. [1].

Among the five categories of RS, the amylose–lipid complex (RS5) has recently emerged as a promising and underexplored type. RS5 forms when the hydrophobic carbon chain of lipids enters the amylose helix, increasing resistance to enzymatic digestion and reducing starch hydrolysis. Existing studies have examined RS5 formation mainly in model starch systems or limited food applications. Still, research on its formation in traditional Southeast Asian foods, many of which naturally combine starch and lipids, remains scarce. This represents a significant opportunity for developing novel functional foods.

Traditional Southeast Asian foods are over 40% carbohydrate-based, with coconut milk as a main ingredient [2]. This phenomenon can potentially form RS5, the amylose-lipid complex. Some main rice-based foods with coconut milk are Sticky Rice in Thailand, Nasi Lemak in Malaysia, and Buras in Indonesia. Pangastuti and Permana [3] studied one RS5 product from Indonesia, Nasi Uduk, a rice dish cooked with coconut

milk. The research shows that Nasi Uduk contains 7-11% RS5. Nasi Uduk is freely gelatinized, so its RS content remains low. These studies collectively indicate that RS5 formation is influenced by amylose content, lipid concentration, and post-cooking processing. However, they do not address how specific rice varieties with different amylose levels interact with lipids to form RS5 in more structurally constrained foods.

Amylose might influence amylose-lipid inclusions because lipids mainly form complexes with the amylose component of starch, while amylopectin barely interacts with lipids. High-amylose rice can form lipid complexes more readily. High-amylose rice has a firmer texture than low-amylose rice, which has a softer texture. One example of a high-amylose rice product is Buras, an Indonesian rice cake. Buras is a traditional Bugis dish widely consumed in Makassar, South Sulawesi, Indonesia. Buras has a unique cooking process: it is wrapped in banana leaves. This method prevents the contents of the wrap from leaking out due to its closed structure, such as coconut milk fat or an amylose fraction from rice, thereby allowing for a more intense amylose-lipid interaction rather than leakage. According to Seo et al. [4], restricting amylose movement strengthens hydrogen bonds, forming a more stable structure that resists digestive enzymes and increases resistant starch content. The main ingredient in making Buras is cooked rice with a firm texture, which helps produce Buras with a dense, easy-to-cut consistency. Among the many rice varieties in Indonesia, three popular ones with a firm texture are Setra Ramos, Rojo Lele, and C4 Super. Firm-textured rice often has an amylose content of >20%. High amylose content is important in the retrogradation process, in which the starch structure reforms after cooking and cooling, creating a more stable structure resistant to digestion and potentially increasing the resistant starch content in Buras.

In addition to amylose content, many researchers are currently seeking to enhance complex formation using physical methods. Another factor that can increase RS content is the process of several cooling-reheating cycles. This process involves heating, which gelatinizes the starch, allowing amylose molecules to stretch, coil, and form new helices with lipids through retrogradation [5]. Despite this, no study has yet examined how different amylose levels from rice varieties influence the formation, structure, physicochemical properties, and digestibility of RS5 within a traditional food matrix such as Buras, especially under repeated cooling–heating treatments.

Thus, the current study addresses this research gap by investigating how rice varieties with differing amylose contents affect the morphology, crystallinity, functional groups, and starch digestibility of amylose–lipid complexes formed in Buras. The findings will contribute to a new understanding of (1) RS5 formation mechanisms in real food systems rather than isolated starch models, and (2) how traditional processing techniques can be optimized to produce functional foods with lower starch digestibility.

II. Material and Methods

A. Material

The primary raw materials for making buras are rice (obtained from the local market in Sleman, Yogyakarta) with the varieties Setra Ramos, Rojo Lele, and C4 Super. Respectively, all rice moisture content was <15% (11.51%, 11.80%, and 11.48%). Commercial coconut milk (Kara, Indonesia) was obtained from Supermarkets in Sleman, Yogyakarta. The additional materials are bay leaves and salt. The chemicals used for analysis include α-amylase (Sigma A3176, Sigma-Aldrich Inc., US), pepsin (Sigma P7000, Sigma-Aldrich Inc., US), and GOD-FS (Ref 1 2500 99 83 021, DiaSys Diagnostic Systems GmbH, Germany).

B. Methods

Buras Making Procedure

The buras were made based on previous research [6]. 100 g of rice is washed with water and added with 200 mL of coconut milk (1:2), 1 bay leaf, and 1 g of salt. Rice and coconut milk are stirred, pre-gelatinized for 20 min, and then cooled. The next step is to place the pre-gelatinized rice in a 30x20 cm banana leaf and tie it. Buras is boiled for 1.5 h and cooled to room temperature (30°C).

Multiple Cooling-Reheating Cycles of the Buras

Cooling-reheating Cycles emphasize society's behavior when food is not completely consumed. It is cooled in the refrigerator and then reheated. This process can be repeated up to 2 times, as was done in this study. The multiple cooling-reheating cycle treatment involves storing the Buras at 4°C for 24 h in a cooling room and reheating them in a microwave at 80-100 °C (medium heat) for 5 min, for 1 cycle (S1). Buras that step into cycle 2 will be stored again at 4°C for 24 h in a cooling room, then reheated in a microwave at 80-100 °C (medium heat) for 5 min; this is for cycle 2 (S2). Meanwhile, the treatment for Buras without a cycle (S0) doesn't involve storage at 4°C for 24 h in a cooling room and repeated heating.

Sample Preparation

To prepare buras into a powder, they are placed in the freezer for 24 h, then transferred to a cup with a small hole. The frozen buras are then put into a freezer dryer for 24 h. After that, the buras are refined and filtered with a 100-mesh sieve.

Measurement of Amylose Content

The analysis was conducted as follows [7] for amylose content: A 0.1 g sample was placed in a 100 mL volumetric flask, and 1 mL of 95% ethanol and 9 mL of 1N NaOH were added. The mixture was heated in boiling water for 10 min, then cooled. Distilled water was added to dilute the mixture to the 100 mL mark. A 5 mL portion of the sample solution was transferred to another 100 mL volumetric flask, followed by the addition of 1 mL of 1 N acetic acid and 2 mL of 0.2% iodine solution. The solution was further diluted to the mark and incubated for 20 min. Then, its absorbance was measured at 625 nm. A standard calibration curve was prepared using known amylose

concentrations (0–4 ppm), and a linear regression equation was generated. The amylose content (%) was then calculated using the following equation:

$$\text{Amylose Content (\%)} = \frac{\text{C} \times \text{V} \times 100}{\text{W} \times (100 - \text{M})} \quad (1)$$

Description:
C: Amylose concentration of the sample (µg/mL), obtained from the standard calibration curve
V: Volume of extract (mL)
W: Weight of the sample (mg)
M: Moisture content (%)

Measurement of Resistant Starch Content

The resistant starch (RS) content was analyzed using the enzymatic method [8]. A 25 mg sample of powdered material was placed in a 15 mL Falcon tube, mixed with 2.5 mL of KCl-HCl buffer, and vortexed. Then, 50 µL of pepsin solution was added, and the mixture was vortexed again, incubated at 40 °C for 60 min, and then cooled. Afterward, 2.25 mL of 0.1 M tris-maleate buffer (pH 6.9) (Sigma A3176, Sigma-Aldrich Inc., US) and 250 µL of α-amylase solution were added, and the sample was incubated at 37 °C for 16 h. The sample was centrifuged at 3000g for 15 min, and the supernatant was discarded. The residue was washed with 10 mL of distilled water, centrifuged again, and the supernatant was discarded. The remaining residue was mixed with 3 mL of distilled water and 0.75 mL of 4 M KOH solution and incubated at 37 °C with constant agitation for 30 min. Next, 1.375 mL of 2 M HCl, 0.75 mL of sodium acetate buffer, and 20 µL of amyloglucosidase enzyme were added, and the sample was incubated at 60 °C in a shaker for 45 min. The sample was centrifuged twice at 3000g for 15 min, and the supernatant was collected into a 25 mL volumetric flask and diluted. For analysis, 0.5 mL of the solution was combined with 1 mL of the GOD solution in a test tube and incubated at 37 °C for 30 min. Finally, the absorbance was measured at 510 nm using a UV-Vis spectrophotometer. The RS content was then calculated using the following equation:

$$\text{RS Content (\%)} = \frac{[\text{Glucose } (\frac{\text{mg}}{\text{mL}}) \times \text{DF} \times 0.9]}{\text{Sample weight (mg)}} \quad (2)$$

Description:
Glucose (mg/mL): Glucose concentration in the sample solution (mg/mL), obtained from the standard curve
DF (Dilution Factor): Total volume of the hydrolysate or sample solution (mL)
0.9: Conversion factor from glucose to starch
Sample weight (mg): Weight of the dry sample (mg)

In Vitro Digestibility

In vitro digestibility was determined using the enzymatic method [9]. A 50 mg sample was combined with 10 mL of KCl-HCl buffer (pH 1.5) and homogenized for 2 min. Then, 0.2 mL of a pepsin solution (1 g of pepsin in 10 mL of KCl-HCl buffer) was added, and the mixture was incubated in a water bath shaker at 40 °C for 1 h. After incubation, the sample was diluted to 25 mL with tris-maleate buffer (pH 6.9), and 5 mL of α-amylase (2.6 IU) was added. The mixture was then incubated at 37 °C in the water bath shaker. A 1 mL aliquot was taken every 30 min from 0 to 3 h and heated at 100 °C for 5 min to inactivate the enzyme, ensuring consistent timing. Following this, 3 mL of 0.4 M sodium acetate buffer (pH 4.75) and 60 µL of the solution were added to each aliquot, and the mixture was incubated at 60 °C for 45 min in the water bath shaker. Each sample was diluted to a final 10-100 mL volume with distilled water. For glucose analysis, 0.5 mL of the solution was mixed with 1 mL of the GOD solution, and the glucose content was converted to starch by multiplying by 0.9. The starch digestion rate was expressed as the percentage of total starch (TS) hydrolyzed over different time intervals (30, 60, 90, 120, and 180 min). The total starch hydrolyzed was then calculated using the equation:

$$\text{Total starch hydrolyzed (\%)} = \frac{\text{Glucose } \left(\frac{\text{mg}}{\text{mL}}\right) \times 0.9}{\text{Sample weight (mg)}} \times 100 \quad (3)$$

Description:
Glucose (mg/mL): Concentration of glucose released in the hydrolysate, calculated from the absorbance value using a standard curve
0.9: Conversion factor from glucose to starch (based on molecular weight ratio)
Sample weight (mg): Dry weight of the sample used for digestion
100: Multiplier to convert the result into a percentage

Scanning Electron Microscope (SEM)

The sample was placed on a holder using double-sided tape, a gold layer was applied, and the sample was then vacuum-conditioned. Then, it was photographed using an SEM (JEOL JSM-6510 LA), at magnifications ranging from 500x to 3,000x and an acceleration potential of 10 kV.

X-ray diffraction (XRD)

X-ray diffraction analysis was performed using a Bruker D8 Advance Eco at room temperature (25°C). The system used a copper anode, with the generator set to 40 kV and 25 mA, and a wavelength of 1.5406 Å for detection. The diffraction pattern was recorded within a 5-35° (2θ) range over 76.80 seconds, with a step size of 0.020° and a time per step of 0.40 seconds.

Fourier-Transform Infrared Spectroscopy (FTIR)

The sample, which weighed 2 mg, was added to 200 mg of KBr (Potassium bromide) and homogenized. Furthermore, the homogenized sample and KBr were made into pellets. Each spectrum was scanned using FT-IR spectroscopy at room temperature with a 4 cm^{-1} resolution within the 500-4000 cm^{-1} range.

Statistical Analysis

The experimental design was a non-factorial completely randomized design (CRD) with three replicates. Data were analyzed using Microsoft Excel 2013 (Microsoft Corporation, USA) and SPSS 23 (IBM, USA), and presented as average values ± standard deviation. The resistant starch content and starch digestibility were analyzed using Univariate ANOVA and post-hoc Duncan's test at the 95% confidence level ($p < 0.05$). Starch crystallinity and functional properties were investigated using OriginPro 2025 (OriginLab Corporation, USA).

III. Result and Discussion

Effect of Amylose Content on Resistant Starch Type 5

Analyzing amylose content is crucial for forming amylose-lipid complexes, as amylose directly interacts with lipids to create a complex structure that is more resistant to digestive enzymes. Higher amylose content enhances the potential for complex formation. The amylose content of each rice variety varies due to cultivation location, soil nutrients, and cultivation methods. Amylose content over 20% is classified as high amylose, while under 20% is classified as medium to low amylose [10]. Table 1 shows that Setra Ramos and C4 Super rice varieties have high amylose levels, with each array exceeding 20%. In contrast, Rojo Lele rice has the lowest amylose content; it showed a significant difference ($p < 0.05$) compared to the other rice varieties.

TABLE 1
AMYLOSE AND RS5 CONTENT BASED ON DIFFERENT VARIETIES OF RICE

Rice Variety	Amylose (%)	Resistant Starch (%)
Setra Ramos	25.14 ± 1.2^{c}	26.54 ± 0.36^{c}
C4 Super	22.46 ± 1.04^{b}	23.32 ± 0.63^{b}
Rojo Lele	17.44 ± 1.92^{a}	19.91 ± 0.32^{a}

The amylose content in rice positively correlates with resistant starch content; higher amylose makes starch more resistant to enzymes, delaying starch hydrolysis [5]. High amylose content also affects starch's thermal properties, raising its gelatinization and retrogradation temperatures. Amylose's long-chain structure forms strong hydrogen bonds with water during gelatinization and retrogradation, creating a tight crystalline structure. Higher amylose content is positively correlated with higher levels of resistant starch. Amylose-lipid complex formation occurs when amylose, a linear glucose polymer with units linked by α-1,4-glycosidic bonds, interacts with lipids. The hydrophobic interior of the amylose helix readily interacts with nonpolar molecules like lipids. In this case, coconut milk is known to contain saturated fatty acids. The most dominant is lauric acid, with a rate of 80.72% [6] using the same coconut milk source. In the presence of lipids such as fatty acids, monoacylglycerols, or phospholipids, the lipid molecules are incorporated into the amylose helix through hydrophobic interactions.

Lipids, particularly those with long hydrocarbon chains, are attracted to the hydrophobic interior of the amylose helix, leading to the formation of inclusion complexes where the lipid molecules are trapped within the helix. Under certain conditions, such as heating, amylose molecules can rearrange into single helices, creating an ideal environment for lipid molecules to fit inside. The interaction between lipids and the helical amylose structure results in stable inclusion complexes, with the lipid's hydrocarbon chain embedded in the amylose's hydrophobic cavity [11]. If present, the lipid's polar head remains outside the complex and interacts with the surrounding water. This amylose-lipid interaction forms a physical barrier that inhibits digestive enzymes from accessing the glycosidic bonds in amylose. As a result, enzymes like α-amylase, which break down starch into simpler sugars, are blocked from acting on the amylose-lipid complex. The complex's crystalline structure further limits enzyme binding and activity, enhancing resistance to digestion. Setra Ramos was chosen for further study due to its amylose and resistant starch content.

In Vitro Starch Digestibility

The *in vitro* starch digestibility of amylose-lipid complexes refers to how well they resist enzymatic breakdown during digestion. Amylose-lipid complexes are formed when amylose, a linear polymer of glucose, interacts with lipids (fats), creating a structure that is less accessible to digestive enzymes such as α-amylase. The digestibility of Buras starch with various rice varieties is shown in **Figure 1**.

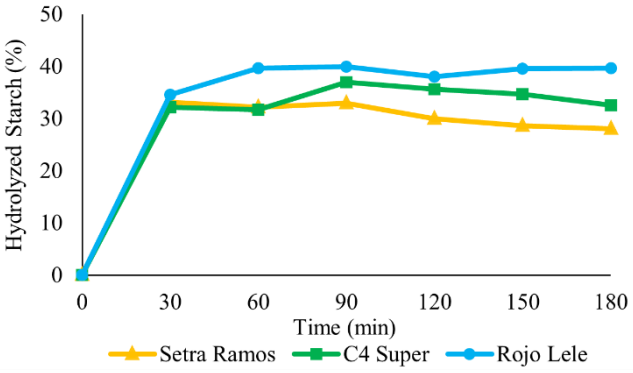


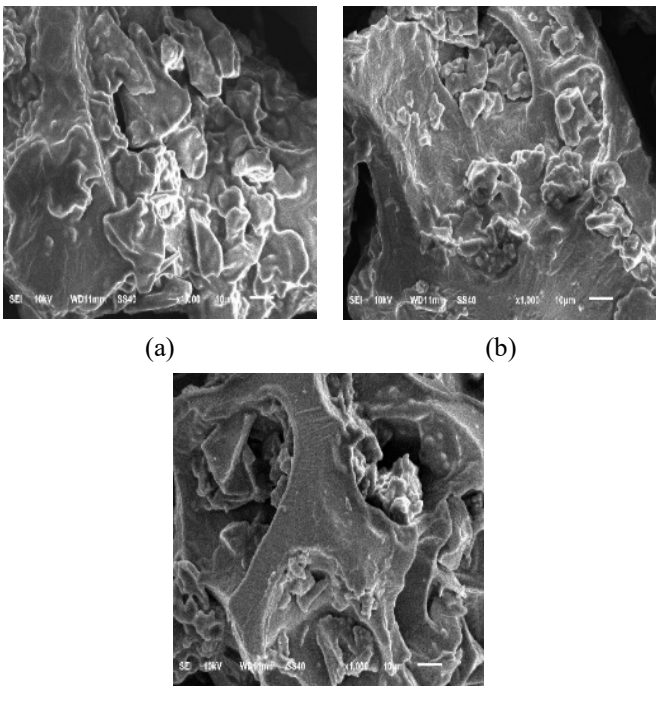
Fig. 1 Starch digestibility of Buras with various rice varieties

Figure 1 shows that Setra Ramos (38.02%) has the most significant decrease in starch hydrolysis at 180 min, followed by C4 Super (42.58%) and Rojo Lele (49.69%). This relates to the resistant starch content (Table 1), which indicates that higher RS5 content is associated with lower starch hydrolysis. The amylose-lipid complex formation mechanism, as a form of RS5, limits α-amylase access to the glycosidic bonds of amylose molecules. Enzymes such as α-amylase bind to starch chains and break bonds between glucose units. Enzymes cannot easily reach or bind to starch chains in a denser crystal structure. Amylose-lipid complex formation decreases the solubility of amylose in water, which is crucial for its enzyme breakdown.

In its original form, amylose is more soluble and more easily gelatinized when heated with water, making it easier for enzymes to hydrolyze. However, after complexing with lipids, it becomes less soluble, further reducing the enzyme's efficiency. Amylose molecules normally have binding sites for α-amylase, which allows the enzyme to attach and catalyze the breakdown of starch into smaller glucose units. However, in the complex, the amylose strands are partially shielded by lipid molecules, which reduces the number of binding sites accessible to the enzyme [12]. Even if the enzyme can bind to some parts of the amylose molecule, the overall rate of hydrolysis is significantly slowed due to the reduced flexibility and accessibility of the amylose strands in the complex.

Morphology of Buras with Scanning Electron Microscope

Scanning Electron Microscopy (SEM) reveals structural changes in Buras granules, including roughness, compactness, or porosity, that occur when amylose interacts with lipids. These morphological details are important for assessing how well lipids integrate into the amylose structure and how they affect the formation of resistant starch. **Figure 2** shows a scanning electron microscope image of the Buras sample (1000×).



(a) (b)

(c)

Fig. 2 SEM images of Buras with different rice varieties: (a) Setra Ramos, (b) C4 Super, and (c) Rojo Lele

Figure 2 shows SEM images taken at 1000× for Buras made from rice of different varieties. As shown, the shape and integrity of each sample are intact, with no visible breaks or cracks, and an average size of 10 μm. The starch granules are damaged by the heating during cooking and the cooling during sample preparation. The addition of coconut milk during Buras preparation causes flakes to form on the granules' surface. This is due to the saturated fatty acids in coconut milk. Saturated fatty acids in coconut milk include lauric, palmitic, myristic, and stearic acids. Saturated fatty acids can solidify on the surface of starch granules. This fatty acid layer from coconut milk can affect the swelling capacity of starch granules during water diffusion. According to Cervantes-Ramírez [13]. The size of starch granules can affect physicochemical properties, including gelatinization and retrogradation, enzyme susceptibility, crystallinity, and solubility.

Based on the different amylose content in Buras, the integrity of the granules can be seen: Setra Ramos > C4 Super > Rojo Lele. Setra Ramos granules maintain the most intact structure, with a smooth surface and precise grain contours. In contrast, C4 Super (Fig. 2b) granules are partially damaged, with some surface wrinkles. Rojo Lele granules are very broken, with more obvious wrinkles and cracks than Setra Ramos and C4 Super. These results indicate that rice with higher amylose content better maintains grain morphology. The mechanism is that amylose molecules interact with amylopectin, thereby limiting swelling and grain damage [14]. Lipids in the complex help retain the structural grain around the granule surface [15]. The higher the amylose content, the stronger the grain integrity, the more amylose-lipid complexes, and the more intact grains even after going through the cooking process.

The morphology of Setra Ramos (Fig. 2a) maintains granule integrity due to its higher amylose content compared to Rojo Lele (Fig. 2c). Higher amylose promotes the formation of dense intermolecular hydrogen bonds within starch granules, increasing their resistance to swelling and damage during processing. The high amylose content also reduces solubility through strong molecular interactions, limiting the granules' capacity to absorb water and swell excessively. During retrogradation, amylose chains readily recombine to form a gel structure that reinforces the granule matrix, enhancing structural stability. In contrast, Rojo Lele's low amylose content results in an uneven lipid coating of the granules, making them more prone to gelatinization. This process facilitates water entry into the granules, compromising their integrity and weakening their structure.

Starch Crystallinity with X-ray diffraction

X-ray diffraction (XRD) reveals changes in crystal structure induced by a specific process. XRD identifies changes in the starch's molecular structure, particularly the formation of crystalline regions. These regions are more resistant to enzymatic digestion, which is crucial in forming resistant starch types like RS5. In this study, changes in the crystal structure were caused by amylose content, lipid addition, and the cooking process, including heating temperature. The amylose-lipid complex can be seen if there is a V pattern in diffraction at 12°, 13°, 18°, and 20°, with a scale of 2θ [16]. **Figure 3** shows the peak angle diffraction of 13°, 17°, and 20° in each variety.

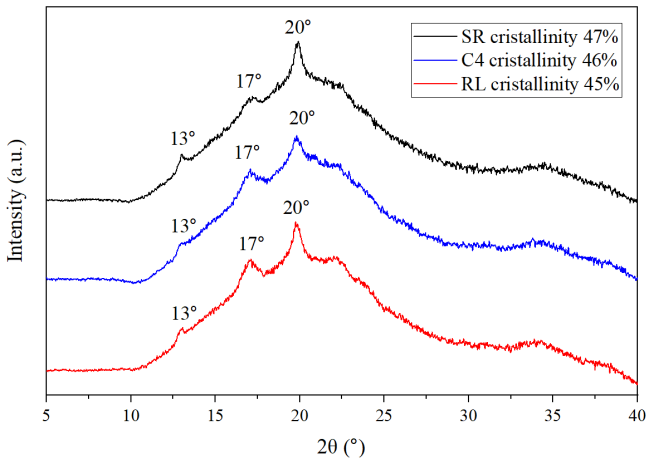


Fig. 3 X-ray diffraction pattern of Buras from different rice varieties

Based on **Figure 3**, all three samples are almost amorphous or semicrystalline. The V-type crystal pattern is formed because a single amylose helix forms a complex with nonpolar compounds such as fatty acids. The formation mechanism is that the nonpolar fatty acid tail enters the hydrophobic core of the amylose helix. Amylose forms a left-handed helix with lipid molecules in the helix cavity; amylose-lipid interactions stabilize this helix complex [17]. This amylose-lipid complex is arranged into a crystal lamella called a V-type crystal and is categorized as a V-type polymorph. A 13° peak represents the lowest peak of amylose-lipid complexation, so a less regular amorphous region characterizes this peak. The 13° peak reflects the arrangement of the amylose helix around the lipid molecule; its intensity can vary with the strength of the complex. The formation of relative intensity indicates the degree of partial crystallinity. In addition, the 17° peak represents the semicrystalline region of the amylose-lipid complex and is associated with the alignment and density of amylose-lipid interactions [18]. The peak angle indicates the balance of amorphous and crystalline regions. The sharper angle means a higher degree of structural order in the complex. Furthermore, the sharpness and intensity of the 20° peak indicate crystallinity in the amylose-lipid complex [19]. The peak at this angle indicates the formation of a well-organized and long-range V-type amylose crystal structure.

Thus, based on Fig. 3, Setra Ramos (SR), with the highest amylose content (25.14%), shows stronger amylose-lipid interactions, forming more helical structures and producing the highest crystallinity (47%). This is evidenced by the sharpness of the 20° peak, which further confirms this high degree of structural organization. In contrast, the lower amylose content of Rojo Lele (RL) produces the least crystalline amylose-lipid complex, as evidenced by the lower intensity of its 17° and 20° peaks, and the peaks tend to broaden, reflecting a less ordered structure. The information that can be derived from diffractogram results in food science is that the high crystallinity observed in amylose-lipid complexes, such as SR, offers several functional benefits, including increased thermal stability, greater resistance to retrogradation, and controlled digestibility. Controlled digestibility in the crystalline region creates a dense, orderly structure that is more resistant to enzymatic hydrolysis. Digestive enzymes such as α-amylase have greater difficulty accessing glycosidic bonds in these crystalline regions than in the more accessible amorphous regions. The V-type crystal structure, formed by amylose-lipid interactions, further limits enzyme penetration and slows digestion. These properties make amylose-lipid complexes with high crystallinity valuable for developing functional foods, such as low-glycemic-index products that offer metabolic and health benefits.

Fourier-transform infrared spectroscopy

Fourier-transform infrared (FTIR) spectroscopy is an effective method for identifying functional groups and changes in chemical bonds and molecular interactions by measuring the absorbance of infrared radiation at specific frequencies. FTIR is frequently employed in conjunction with X-ray Diffraction (XRD) to validate the presence of V-type crystallinity [20]. XRD offers direct insights into the crystalline structure by detecting characteristic diffraction peaks, typically observed at 13°, 17°, and 20°, which signify the ordered arrangement of amylose-lipid complexes. Meanwhile, FTIR complements this analysis by identifying the molecular interactions that underpin the formation of these crystalline structures, such as hydrogen bonding and amylose-lipid interactions, providing a comprehensive understanding of the material's structural and chemical properties. **Figure 4** shows the infrared spectrum scan of the shift or change in spectral band intensity associated with certain functional groups on RS5.

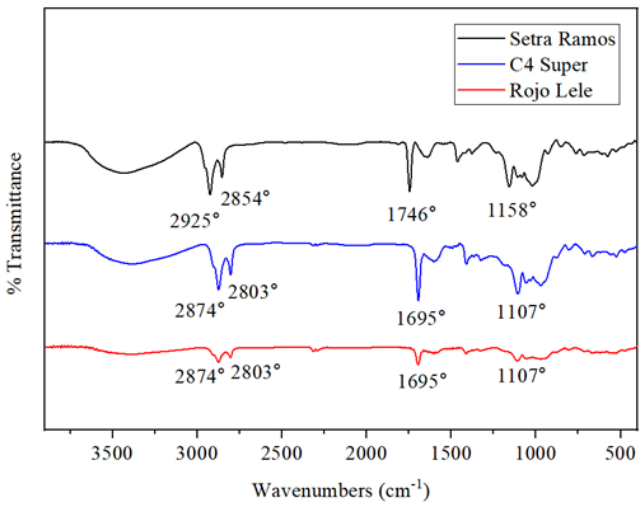


Fig. 4 FTIR spectra pattern of Buras from different rice varieties

The peaks observed in **Figure 4** in 2900-3000 cm^{-1} are due to the stretching vibrations of the CH_2 and CH_3 groups of aliphatic compounds. The peak at 2925 cm^{-1} was observed in Setra Ramos and was much more prominent than in the other samples. This indicates a stronger interaction between amylose

and lipids, contributing to increased crystallinity and the formation of resistant starch. The C–H groups help bind lipid molecules in the amylose helix. Although the hydroxyl groups are polar, amylose can wrap around the hydrophobic regions of lipid molecules (such as fatty acid chains), minimizing water interactions and facilitating complex formation. The stretching region at C=O (1700-1800 cm^{-1}) is related to the stretching vibrations of the carbonyl bonds in the ester or lipid functional groups. A dipole–dipole interaction involving the carbonyl oxygen of the polar functional group and the hydroxyl groups in amylose contributes to stabilizing the amylose helix [16]. When the carbonyl group is connected to the carboxyl (–COOH) or ester (–COOR), the carbonyl group can also function as a hydrogen bond acceptor with the hydroxyl group of amylose, thereby increasing the stability of the complex. Setra Ramos shows a stronger peak at 1746 cm^{-1} in this region, indicating a more complex amylose-lipid interaction that forms type V crystals. The region around 1150-1200 cm^{-1} is associated with the stretching vibration of C–O bonds in the glycosidic bonds of starch.

V-type crystals are formed when the amylose helix wraps around the lipid molecule, producing unique vibrational frequencies, especially in the C–H and C=O regions. Strong hydrogen bonds within the amylose helix and between amylose and lipids also contribute to this structure. FTIR detects changes in the O–H stretching region due to these bonds. FTIR analysis, which showed the Setra Ramos peaks in the C–H and C=O regions, yielded the sharpest peaks, consistent with the higher crystallinity of the cationic starch silica observed in the XRD data. This high crystallinity was enhanced by lipid melting, resulting in a more pronounced and stable amylose polymer. On the other hand, Rojo Lele showed higher, softer peaks, indicating the presence of many amorphous regions, resulting in a low crystal content and a weak reversion of lipid melting into an amylose polymer. The sharpness of the FTIR peaks is associated with crystallinity, molecular interactions, sample purity and preparation, and the instrument used, including its resolution and sensitivity.

The Effect of Cooling-Reheating Cycles on Resistant Starch Content and Starch Digestibility

Setra Ramos is selected for further study because it has the highest amylose and RS5 content, which contribute to reduced starch digestibility. Resistant starch type 5 develops as gelatinized starch retrogrades or recrystallizes, especially its amylose component. As gelatinized starch recrystallizes into a more ordered structure, its resistance to pancreatic amylase increases. RS3, a physically modified resistant starch, forms more readily during repeated heating and cooling cycles, with each cycle increasing the RS content in Buras. Physical modification via heating is particularly effective in increasing RS content in most starches, as it causes the starch granules to swell, mobilizing starch polymers and facilitating the separation of crystallized amylose domains upon cooling. The relationship between the increase in RS content and repeated cooling-reheating cycles on the digestibility of Buras is shown in **Figure 5**.

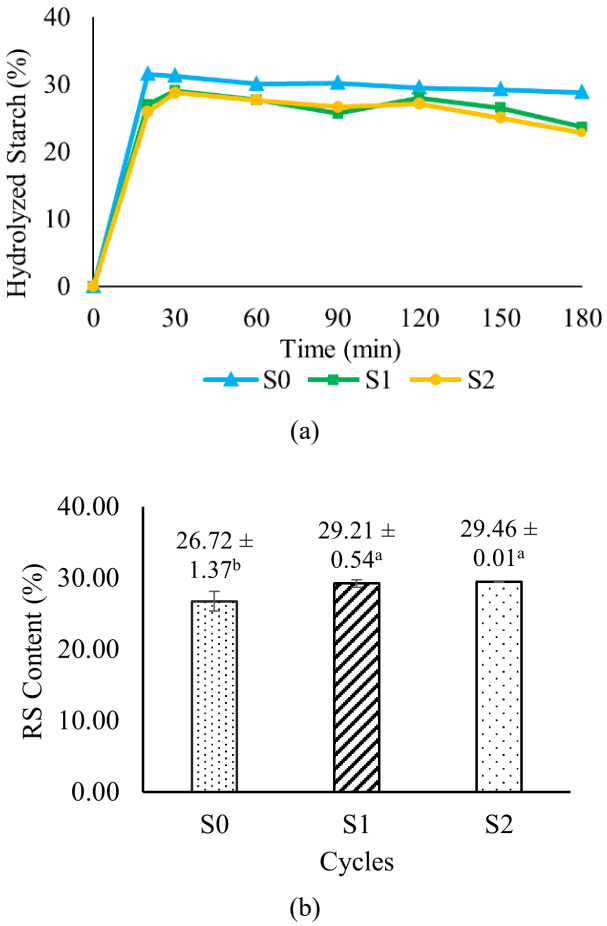


Fig. 5 (a) Resistant starch content of multiple cooling-reheating cycles, and (b) Hydrolyzed starch of multiple cooling-reheating cycles. Where: S0 = no cycle; S1 = 1 cycle; S2 = 2 cycles.

In one cycle (S1), the cooling-reheating process increases resistant starch content significantly ($p < 0.05$) compared to that which does not go through cooling-reheating (S0). The heating stage breaks down starch granules, releasing amylose chains and interacting with lipids. Cooling promotes retrogradation, where amylose chains realign and form V-type crystal structures that resist enzymatic hydrolysis. The sharp increase in RS content demonstrates the effectiveness of a single heating-cooling cycle in promoting structural reorganization and crystal formation in starch. Cooling-reheating causes the amylopectin double helix structure to dissociate hydrogen bonds, melt crystallites, and release amylose fractions from granules [21]. These amylose fractions form a double helix structure with lipids and bind to other double helices to form crystallites. Amylose has a linear chain structure with α1-4 glycosidic bonds, making its molecules more flexible and able to form a tight linear helix, which α-amylase cannot access. In

contrast, amylopectin has a branched structure with α-1,4 and α-1,6 glycosidic bonds.

During the first heating-cooling cycle (S1), amylose chains are released from starch granules during gelatinization and interact with lipid molecules. Cooling aligns these amylose chains into V-type crystal structures, forming resistant starch (RS5). After this initial cycle (S1), most available amylose interacted with lipids, forming stable complexes and reaching saturation. The V-type crystals formed in the first cycle (S1) are thermodynamically stable, and additional heating-cooling cycles (S2) do not significantly change the structure or increase crystallinity. This aligns with the research by Anugrahati et al. [22], which used rice with added coconut milk, also known as 'Nasi Uduk', where one cycle of cooling-reheating (S1) increased the RS content of Nasi Uduk by 5.61%. In contrast, from S1 to the second cycle (S2), it was only 0.54%. This is because RS formation depends on the availability of free amylose. After the first cycle, most of the released amylose retrogrades and is incorporated into resistant crystalline structures. Without additional amylose, further cycles provide little substrate for additional RS formation, leading to stagnation [23]. As the number of amorphous regions decreases, the potential to form more RS also declines. Repeated heating-cooling cycles can introduce thermal stress, potentially disrupting V-type crystals already formed and reducing the net RS content. Optimizing RS formation requires careful control of processing conditions such as heating, cooling, and storage, using high-amylose starches, and adding lipids or other stabilizing agents.

IV. CONCLUSION

RS5 has been reported to reduce postprandial glucose response by limiting starch hydrolysis and enhancing colonic fermentation. The Setra Ramos rice variety used in Buras has the highest amylose and RS5 content, at 25.14% and 26.54%, respectively. This indicates that higher amylose content is associated with higher RS5 content. Therefore, Setra Ramos was evaluated at the physical modification stage. Physical modification through heating and cooling cycles increases retrogradation, resulting in higher resistant starch formation and lower digestibility. The best conditions are achieved with a single heating-cooling cycle (S1), which significantly increases resistant starch by 9%. The increased RS5 content in Buras suggests its potential as a functional carbohydrate source for glycemic control, particularly for individuals with impaired glucose tolerance or at risk for type 2 diabetes. From a technological perspective, this study shows that the cooling-reheating method can be applied to other traditional Indonesian products containing starch to increase RS, lower glycemic index, and help reduce consumers' risk of diabetes.

ACKNOWLEDGMENT

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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