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The Prebiotic Index of Dried-*Growol* Made with Different Cassava Varieties and Cooking Methods

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Abstract

Cassava is a staple food source of carbohydrates that contain high starch levels. However, low amylose starch consumption can result in increased postprandial blood sugar. Therefore, it is necessary to provide food based on cassava, such as dried-*growol*, which gives a feeling satiety for a long time and has a prebiotic effect. This research aimed to evaluate the effects of cassava varieties and cooking methods on the resistant starch (RS) content and the prebiotic index of dried-*growol* produced. This research was carried out in a completely randomized factorial design with two factors, namely cassava variety (M = *Mentega*, L = *Lanting*, and K = *Ketan*) and dried-*growol* cooking method (Au = Autoclave, St = Steaming, and PC = Pressure cooker). Dried-*growol* was processed through preparation, fermentation, boiling, cooling, and drying. The cassava and dried-*growol* were analyzed for their moisture, starch, amylose, and RS content, while the prebiotic index was analyzed on dried-*growol*. Prebiotic index testing used two cultures of lactic acid bacteria: *Lactobacillus rhamnosus* and *Lactobacillus plantarum*. The results showed that RS levels were only influenced by cassava varieties. Dried-*growols* from *Lanting* variety, cooked with steaming (L-St) and with a pressure cooker (L-PC), contain high RS, ranging between 22.51 and 27.03 g 100 g^{-1} dry matter, and have potential as prebiotic food as indicated by the increased viability of *L. rhamnosus* and *L. plantarum* bacteria grown in media with cooked dried-*growol* supplements of L-St or L-PC, with a prebiotic index between 0.82 and 0.90. Thus, dried-*growol* has the potential to be a functional prebiotic food that can serve as a staple food that is beneficial for health.

Keywords: dried-*growol*; functional-food; prebiotic; resistant-starch; retrogradation

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INTRODUCTION

Currently, the primary staple food source of carbohydrates in Asia is rice. As a staple food, the demand for rice is increasing, as indicated by the high demand compared to the production. Moreover, the need is predicted to increase by 60% in 2030 (Rozi et al., 2023). If this condition is left unchecked, it can lead to a food crisis. Indonesia ranked fourth with rice consumption of 35.3 million tons $year^{-1}$ (Rahayu, 2023), while rice production in 2023 was only 31.10 million tons (Statistics of Indonesia, 2024). Rice consumption has reached 114.60 kg $capita^{-1}$ $year^{-1}$ (National Research and Innovation Agency of Indonesia, 2022). Therefore, alternative staple foods are needed to overcome the rice production and demand imbalance.

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Cassava is a food source of carbohydrates in several regions in Indonesia. Cassava contains high starch, so it has the potential to be used as a food source of carbohydrates to replace rice. National cassava production reached 16,764,227 tons in 2023 (Directorate General of Food Crops, 2023) with an average availability of 58.55 kg capita^{-1} year^{-1}, while consumption of cassava only reached 5.86 kg capita^{-1} year^{-1} (Mas'ud and Wahyuningsih, 2023). Thus, it is necessary to diversify food sources of cassava-based carbohydrates, increasing interest in consumption and providing superior health benefits.

There is a dynamic increase in income from cassava consumption, which is greater than that of other carbohydrate sources. This condition shows that tubers can be used as food ingredients in the future. Matita et al. (2024) showed that the processing of wet noodles with modified cassava flour (MOCAF) substitution (80:20) and chia seed flour (*Salvia hispanica*) 10 to 15% as a source of non-gluten vegetable protein can produce wet noodles with texture characteristics that are not different from wet noodles from 100% wheat. Richirose and Soedirga (2023) have also utilized cassava to make gluten-free biscuits with cassava and yam flour ratio formula of 90:10, and the resulting biscuits have good characteristics. Meanwhile, Martínez et al. (2024) used cassava flour fermented with *L. plantarum* for 32 hours to make white bread, and substituting 20% fermented cassava flour could produce white bread with properties that were not different from wheat.

However, fermentation can produce a preferred flavor and aroma, reduce antinutrients such as phytic acid and cyanide acid, and increase protein (Halake and Chinthapalli, 2021). In Indonesia, research on fermented cassava has been conducted on *growol*, a cooked-fermented cassava used as a staple food in several regions of Indonesia, especially in the Special Region of Yogyakarta. *Growol* has a sour taste due to four days of fermentation, resulting in a total lactic acid bacteria of 1.5x10^{6} CFU g^{-1} (Afrianto and Wariyah, 2020). *Growol* is an intermediate moisture food that can only be stored for 3 to 4 days. Therefore, drying has been carried out to increase shelf life, and processing modifications have been made to increase its potential as a source of dietary fiber.

The dried-*growol* processing uses the local *Martapura* variety of cassava, and the cooking uses a two-cycle autoclave with cooling in a refrigerator. The results showed that resistant starch levels of dried-*growol* reached 16.55 to 17.04 g 100 g^{-1} dry matter (Wariyah et al., 2019). Resistant starch is the part of starch that cannot be broken down enzymatically into glucose (Sullivan et al., 2017). Naturally, cassava contains resistant starch between 4.65 and 6.99 g 100 g^{-1}, most of which are included in the RS2 category (Hasmadi et al., 2021). Resistant starch can result from retrogradation of gelatinized cassava starch that occurs during processing. According to Jyothsna and Hymavathi (2017), the resistant starch fraction produced from amylose is included in RS3, so with increasing amylose, the RS3 produced will be greater. The problem is that there are several varieties of cassava, and each variety has different starch and amylose contents (Nurdjanah et al., 2020; Asare and Darfour, 2024), so when processed, it does not necessarily produce dried-*growol* with the same resistant starch content. Zhang et al. (2024) found that in the fermentation of proso millet (*Panicum miliaceum* L.) flour using *L. plantarum* for 24 to 120 hours, there was an increase in lactic acid, which was indicated by a decrease in pH to 3.46. In this fermentation, there was an increase in amylose from the hydrolysis of proso millet starch on days 4 to 5.

Kaur et al. (2023) reported that boiling wheat could increase resistant starch, and according to Karunarathna et al. (2024), boiling chickpeas for 45 minutes and pressure cooking for 15 minutes increased resistant starch by 29% and 41%, respectively. RS3 is retrograded-gelatinized starch, which in *Canna edulis* starch is formed by hydrolyzing the starch, adding water, and then heating under high pressure to produce a paste. The paste is cooled at 4 °C for 12 hours to produce RS3 (Zhang et al., 2021). Kotatha et al. (2023) found that RS3 can be obtained from cassava pulp by retrograding at 4 °C for 24 hours. The increase in RS3 is shown by increasing the total dietary fiber (TDF) from 47.40 to 59.40%, which increases in TDF correlate with the formation of resistant starch.

Resistant starch is a fermentable fiber that can stimulate the growth of probiotic bacteria and produce short-chain fatty acids (SCFA) such as butyric, acetic, and propionic acids, while also reducing fecal pH. Hence, resistant starch is part of prebiotics (Valcheva and Dieleman, 2016). Prebiotics are indigestible carbohydrates that can selectively stimulate the growth and activity of probiotic bacteria, often found in the intestine, by fermenting carbon sources and suppressing the growth of pathogenic bacteria (Reza et al., 2016).

Putri et al. (2012) found that the dominant *Lactobacillus* bacteria growing in *growol* were *L. plantarum* and *L. rhamnosus*. Meanwhile, Yan et al. (2024) stated that the response of the large intestine microflora to the prebiotic and bacterial viability was not the same. In potato-resistant starch, *L. plantarum* bacteria grew higher in the intestine than in glucose (Wang et al., 2022). Therefore, this study aims to evaluate the effect of cassava varieties and cooking methods on resistant starch levels and the growth of probiotic bacteria in media supplemented with cooked-dried *growol*.

MATERIALS AND METHOD

Materials

The essential ingredients used in this research are local cassava (*Manihot esculenta* Crantz) varieties (*Mentega*, *Lanting*, and *Ketan*), purchased from farmers in Kokap, Kulon Progo, Special Region of Yogyakarta, Indonesia. The *Mentega* and *Ketan* varieties are sweet cassava, while the *Lanting* variety is slightly bitter, all three of which are harvested at 8 to 10 months. The physical characteristics of the *Mentega* variety are that the tubers are oval, the outer skin is brown, and the tubers are yellow. The *Lanting* variety has a long round tuber, the outer skin color is greenish brown, and the tubers are white. In contrast, the *Ketan* variety has the characteristics of an oval tuber and dark brown outer skin, and the color of the tubers is milky white (cream).

Chemicals for analyzing starch and amylose content with pro analysis qualifications from Merck, while for prebiotic index testing using MRS Broth and MRS Agar from Merck (Merck KgaA, 64271, Darmstadt, Germany). The probiotic bacterial cultures used were *L. plantarum*, or newly named *Lactiplantibacillus plantarum* (FNCC 0027) and *L. rhamnosus* or with new naming *Laktataseibacillus rhamnosus* (FNCC 0052), which were purchased from the Food and Nutrition Culture Collection (FNCC), IUC Food and Nutrition, Universitas Gadjah Mada, Yogyakarta, Indonesia.

Methods

Dried-*growol* as a prebiotic was processed using the process by Wariyah et al. (2019), with modified treatment at the cooking stage. Next, each cassava variety was processed into dried-*growol* in the following stages: peeling, washing, and cutting across ±5 cm; soaking/ spontaneous fermentation in water for 24 hours with a cassava/water ratio of 1:3; chopping; and pressing to achieve a moisture content of around 55 to 60%. The cassava was then cooked with variations using an autoclave (Pressure sterilizer AI American model 1925x, USA) (at a temperature of 121 °C for 15 minutes), steamed with a steamer (Bima D30, Indonesia) for 15 minutes, and cooked with a pressure cooker (Philips HD2136, China). The *growol* was allowed to cool at room temperature, cooled in a refrigerator (Sharp FRV-300, Indonesia) at a temperature of 4 to 7 °C for 24 hours, then dried using a drying oven (Memmert DIN 40050 IP 20, Germany) at a temperature of 50 °C to a moisture content of 10 to 12%. A rice cooker (Cosmos CRJ-101) was used to cook the dried-*growol*, an incubator (Memmert D06061 Model 500, Germany) was used to test bacterial growth, while a spectrophotometer (Shimadzu mini 1240 Spectrophotometer, Japan) and a set of glassware (Iwaki glass) were utilized for the test chemistry and microbiology.

The analysis carried out on fresh cassava for each variety included measuring moisture content using the static gravimetric method, starch content using the Direct Acid Hydrolysis method (AOAC, 2005), amylose content using the colorimetric method (Williams et al., 1970), and resistant starch content using enzymatic methods (Goni et al., 1996). Dried-*growol* samples from each cassava variety and cooking method were analyzed for moisture, starch, amylose, and resistant starch content and tested for prebiotic index using glucose as a comparison (Palframan et al., 2003).

Determination of dried-growol prebiotic index

The prebiotic index of dried-*growol* was determined *in vitro*, referring to Reza et al. (2016), by fermentation using probiotic microbial cultures, namely *L. plantarum* and *L. rhamnosus*. According to Reza et al. (2016) with modification, each bacterial culture was prepared in MRS Broth with a concentration of 10^{-9}, then diluted again to $1x10^{-5}$ CFU, and 1 ml was taken to be grown in MRS Agar media, supplemented with 3% cooked-dried-*growol*. Dried-*growol* was cooked using a rice cooker (Cosmos CRJ-101, Indonesia) with a dried-growol-to-water ratio of 1:1.9. Each type of probiotic bacteria was incubated in an incubator (Memmert D 0606, Model 500, Germany) at 37 °C under aerobic conditions for 24 hours, and the number of bacterial colonies was counted at 0 and 24 hours. A 3% glucose supplement was used as a comparison. Bacterial growth was determined using the microdilution method. The prebiotic index was calculated based

$$\text{Prebiotic index} = \frac{\text{The number of probiotic bacteria in prebiotics}}{\text{Number of probiotic bacteria in carbohydrates (glucose)}} \quad (1)$$

on bacterial growth for 24 hours, referring to Palframan et al. (2003) (Equation 1).

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Experimental design

The experimental design used was a factorial completely randomized design with two factors, namely cassava variety (*Mentega*, *Lanting*, and *Ketan*) and method of cooking the *growol* (autoclave, steaming, and pressure cooker) with three replicates.

The statistical analysis employed in this study involved one-way analysis of variance (ANOVA), and the data are presented as the mean ±standard deviation (SD). Differences between treatments were tested using the F-test, and then if there were differences, Duncan's multiple range test was used for pairwise comparisons between samples.

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RESULTS AND DISCUSSION

Chemical properties of cassava

The main carbohydrate component in cassava is starch, the content of which is greatly influenced by the variety and harvest age. According to Nurdjanah et al. (2020), the cassava starch content of three cassava varieties (*Manalagi*, *Mentega*, *Krembi*) harvested at the age of 7 to 9 months reached 12.97 to 17.52%, while the amylose content was between 7.22 to 10.35%. In these varieties, the water content was between 62.12 and 66.70%. Table 1 shows that the moisture and starch contents of *Mentega*, *Lanting*, and *Ketan* cassava varieties are significantly different. In contrast, the contents of the amylose and resistant starch are not significantly different. Cassava varieties *Ketan* has the highest moisture content compared to *Mentega* and *Lanting*. Previous research has shown that the moisture content of varieties *Ketan* is indeed higher than that of *Lanting* (Afrianto and Wariyah, 2020). The moisture content of cassava for each variety is different. In the NR variety, it is reported that the moisture content is 63.33±7.77% (Nwachukwu and Simonyan, 2015), almost the same as the moisture content resulting from this study. Likewise, the levels of cassava starch are significantly different.

Ariani et al. (2017) reported that cassava starch content is 19.13 to 24.49%, or around 41.32 to 62.60% (db). These differences can be caused by variety, harvest age, and growing location. The cassava used in this research was 10 months old and came from hilly areas in Kulon Progo Regency, Special Region of Yogyakarta. At this age, the starch content of the Adira-4 variety is around 15.5% (grown with stress due to drought) and 20.40% (grown in optimal water) (Wahyuni and Noerwijati, 2021). According to Mutiara and Bolly (2019), the survey results show that cassava productivity and quality are determined by soil fertility. In addition, the primary cause of declining harvest yields is associated with decreased rainfall and poor soil, which requires a strategy in land management to increase fertility (Mesele et al., 2024).

Based on the results of this study, the amylose content of cassava showed no significant difference. The amylose content of several cassava varieties is between 7.22% and 10.35% (wb) or around 18.66 to 26.32% (db) (Nurdjanah et al., 2020). This result is because there are wide varieties of cassava whose composition variations are greatly influenced by several factors, such as where they are grown and the age at which they are harvested. Likewise, the resistant starch content was similar. Ogbo and Okafor (2015) found that the resistant starch content of natural cassava is between 5.70 and 7.07 g 100 g^{-1} material, while Wariyah et al. (2019) found that the resistant starch content of the local *Martapura* variety of cassava ranged from 8.89 to 9.95 g 100 g^{-1} dry matter.

Chemical properties of dried-*growol*

The chemical composition of dried-*growol* includes water, starch, amylose, and resistant

Table 1. Moisture content, starch, amylose, and resistant starch of fresh cassava

Cassava varieties	Moisture (%)	Starch (% db)	Amylose (% db)	RS (g 100 g^{-1} dry matter)
M	62.14±7.33[a]	70.01±3.50[a]	16.18±1.38[a]	13.70±1.20[a]
L	62.46±2.84[a]	76.33±1.94[b]	15.45±2.30[a]	13.01±0.99[a]
K	69.46±1.60[b]	75.28±5.09[b]	16.45±1.61[a]	14.44±1.38[a]

Note: The different letter behind the number in the same column shows that it is significantly different ($p < 0.05$). Description of abbreviations: M = *Mentega*, L = *Lanting*, K = *Ketan*, RS = Resistant starch

starch, as presented in Table 2. In line with its raw material, cassava, dried-*growol* has a high content of starch, amylose, and resistant starch. The high carbohydrate content makes dried-*growol* suitable for use as a staple food. With a high resistant starch content, dried-*growol* can provide health benefits.

Moisture content of dried-growol

Drying plays an essential role in the dehydration process of various types of food, especially for preserving food with high water content. In the drying process, proper control during drying can produce food with good quality (Guo et al., 2024). *Growol*, as an intermediate moisture food, is easily damaged. With drying, free water in *growol* will be reduced, so that water activity is reduced and food becomes durable. Table 2 shows that the moisture content of dried-*growol* is not significantly different. The moisture content of dried-*growol* is set to less than 12%. The research results showed that the moisture content of dried-*growol* was between 9.44% and 10.26%. Analogous to the moisture content of rice as a staple food source of carbohydrates, Indonesian National Standard states that the moisture content should not be more than 15%. Previous studies have shown that the water content of dried-*growol* is 12.74±1.24% (Wariyah et al., 2019), while the analogue of dried-*growol* is dried-cassava, which has a water content of 9.50±0.45% (Armah et al., 2024).

Starch and amylose content of dried-growol

Starch is cassava's main component, composed of amylose and amylopectin. The starch and amylose content of cassava is influenced by the location where it is grown. Hasmadi et al. (2021) stated that the starch content of cassava harvested at the age of 9 months and planted in the Tawau and Samporna areas in Malaysia, with the starch content of 51.77±0.07 and 61.21±0.17 g 100 g^{-1}, respectively. At the same time, the amylose content was 13.87±0.05% (w/w) and 23.16±0.52% (w/w), respectively. This study used cassava with the *Ketan*, *Lanting*, and *Mentega* varieties from Kulon Progo Regency, Special Region of Yogyakarta, Indonesia, with different compositions (Table 1). The composition of these varieties after being processed into dried-*growol* can be seen in Table 2. Compared with the fresh cassava (Table 1), the starch and amylose content of dried-*growol* was lower than that of the raw material. *Growol* is produced through a fermentation stage by soaking cassava in water for 24 hours under aerobic conditions. During fermentation, the hydrolytic amylolytic bacteria *Lactobacillus* sp. grows, capable of hydrolyzing starch to produce glucose and other soluble carbohydrates (Putri et al., 2012). At the washing stage, the hydrolysis products dissolve and are included in the fermented cassava washing water to lower the starch content. Fermentation of cassava and washing of fermented cassava were carried out under the same conditions, using five parts of water with two washing cycles.

However, the quantitative changes in chemical components were not consistent and did not follow a linear pattern with the chemical content of fresh cassava. The starch and amylose content of fermented cassava is greatly influenced by the cassava variety, as the response to the various fermentation conditions of each array, and the chemical composition of cassava differs for each variety (Afrianto and Wariyah, 2020). Zhang et al. (2024) found that during the fermentation of

Table 2. Moisture, starch, amylose, and resistant starch content of dried-*growol*

Cassava varieties	Cooking method	Moisture (%)	Starch (% db)	Amylose (% db)	RS (g 100 g^{-1} dry matter)
M	Au	9.95±0.25[a]	56.73±2.99[cd]	17.13±2.42[de]	-
	St	9.81±0.85[a]	54.21±4.14[cd]	17.69±2.53[de]	21.32±3.56[a]
	PC	9.55±0.14[a]	58.91±5.08[d]	15.10±2.81[abcd]	20.58±1.75[a]
L	Au	10.26±1.21[a]	48.74±2.89[ab]	17.04±2.11[cde]	-
	St	9.70±0.13[a]	49.30±1.88[ab]	15.06±1.76[bcde]	22.51±2.21[a]
	PC	9.71±0.22[a]	46.84±3.17[a]	13.76±1.11[abc]	27.03±1.90[a]
K	Au	9.78±0.98[a]	46.95±3.27[a]	18.25±2.19[e]	-
	St	9.92±0.50[a]	52.21±3.71[bc]	12.41±1.16[a]	17.30±2.49[a]
	PC	9.44±0.43[a]	51.38±5.45[bc]	13.63±0.11[ab]	19.07±1.97[a]

Note: The different letter behind the number in the same column shows that it is significantly different ($p < 0.05$). M = *Mentega*, L = *Lanting*, K = *Ketan*, Au = Autoclave, St = Steaming, PC = Pressure cooker, RS = Resistant starch. Dried-*growol* from M, L, and K varieties cooked in an Au, preliminary research results show that the resistant starch value is low, so it was not analyzed further non-waxy proso millet starch using *L. plantarum*, the pH of the solution decreased on the first and second days, indicating the formation of lactic acid, which was marked by a reduction in pH and viable cell number. The amylose content of proso millet starch decreased from 21.1±0.72 to 20.6±0.04% but increased again on the third day, suggesting that the lower pH levels are associated with fewer viable bacteria. The fermentation process in this study was carried out for 24 hours, indicating a high potential for amylose reduction.

The cooking method and cassava variety influenced starch and amylose levels in dried-*growol* samples (Table 2). In all processing methods, the starch content of the *Mentega* variety was higher than the *Lanting* and *Ketan* varieties, while the amylose content of the *Ketan* variety was the lowest. The chemical characteristics of each cassava variety, due to the fermentation and washing process, differed in their effects on starch and amylose content. The amylose content increased compared to the base material. Changes in amylose levels in dried-*growol* are caused by starch hydrolysis during fermentation, which can produce straight chains of amylose (Putri et al., 2012). Table 2 shows that the increase in amylose mainly occurred in dried-*growol* M-Au, L-Au, and K-Au. In dried-*growol* M-St, M-PC, L-St, and L-PC, the amylose content is relatively constant, except for the *Ketan* variety. According to Wariyah et al. (2019), the amylose content remains unchanged or decreases after processing into dried-*growol*, which is related to the formation of resistant starch.

Resistant starch content of dried-growol

Cassava naturally contains resistant starch which is resistant to digestion in the small intestine. According to Hasmadi et al. (2021), resistant starch in cassava is RS2, which is granular starch physically covered by solid cell walls, making it difficult to digest. However, the processing method can change RS2 into a different type of resistant starch beneficial for consumption. Liu et al. (2020) found that processing high amylose corn starch (RS2) using a temperature of 120 to 140 °C and then storing it at room temperature for 30 minutes can reduce enzyme resistance. However, if the starch is cooled for 2 hours, retrogradation occurs, resulting in increased resistance to digestive enzymes due to the formation of RS3. Meanwhile, Conde et al. (2022) studied the effect of three treatment factors, including high pressure, cassava flour concentration, and cooking time. The results showed that higher pressure in the processing reduced the percentage of crystalline structure to amorphous. This structural change causes an increase in gelatinization and a decrease in gelatinization enthalpy, resulting in a higher rate of rapidly digestible starch and decreased resistant starch fraction.

Table 2 shows cassava varieties influenced the resistant starch content of dried-*growol*, while the interactions between varieties and cooking methods were not significantly different. Using a high-pressure cooker damages the integrity of the cell walls, causing starch gelatinization to occur quickly (Leite et al., 2017). When gelatinized starch is cooled, it undergoes retrogradation, and retrograded starch is classified as RS3. It was further reported that native cassava starch with a resistant starch content of 2.4±0.2%, resistant starch increased to 28.30±1.0% after debranching using pululanase and heating in an autoclave at 60 °C for 15 minutes (Lertwanawatana et al., 2015). Abioye et al. (2018) stated that cooking cassava starch from two Nigerian cassava varieties (TMS 30572 and 98/0581) by steaming or high pressure combined with cooling in a refrigerator can increase resistant starch to around 5.68 to 6.01 g 100 g^{-1}. However, the effectiveness is higher if the preparation uses isoamylase debranching enzyme to improve amylose so that the resistant starch is between 18.25 and 19.55 g 100 g^{-1}, which represents an increase of 73.00 to 78.00%.

In this study, steaming and pressure cooking were able to increase resistant starch, with the *Lanting* variety showing the highest increase of resistant starch content at around 82.78%, while the *Ketan* variety exhibited the lowest increase at 25.90%. Even without isoamylase, the relatively high increase in resistant starch is probably due to the fermentation process in dried-*growol* processing. According to Putri et al. (2012) during cassava fermentation, starch hydrolysis occurs, which can increase amylose. However, the increase in amylose is influenced by the strain of *Lactobacillus* bacteria that grows during fermentation.

Bacterial viability and dried-*growol* probiotic index

Bacterial viability

Prebiotics are a group of biological nutrients that can be degraded by microflora in the gastrointestinal tract, especially *Lactobacilli* and *Bifidobacteria* (Bamigbade et al., 2022). All prebiotics are included in dietary fiber, but not all dietary fibers are prebiotics. The components of dietary fiber are non-starch polysaccharides (cellulose, hemicellulose, pectin, inulin, and fructans), non-digestible oligosaccharides (fructooligosaccharides, galactooligo-saccharides), lignin, a phenylpropane polymer, and resistant starch. In resistant starch, the prebiotic effects of several varieties are not the same, but all types have positive effects (Tekin and Dincer, 2023). RS3 is a retrograded starch that has been proven to be prebiotic because of its activity in triggering the growth of probiotic bacteria, such as *L. plantarum*, in the colon so that it is fermented to produce short-chain fatty acids (SCFA). Meanwhile, Liang et al. (2023) showed the results of *in vitro* and *in vivo* experiments to determine intestinal microbiota's metabolic response and population to RS3. A study used the strain *Lactiplantibacillus plantarum 84-3 (Lp84-3)*, which can stimulate the production of acetate, propionate, and butyrate from RS3 in the intestine and increase the content of *Lactobacillus*. Increasing SCFA is beneficial because it helps reduce the degree of acidity in the colon and suppress the growth of harmful bacteria.

Figure 1 shows the bacterial growth of *L. plantarum* and *L. rhamnosus* at the beginning (0 hours) and after 24 hours of incubation. The research results showed that the bacterial growth pattern was almost the same, namely increasing at the 24th hour. *L. rhamnosus* bacteria exhibited high growth when supplemented with cooked-dried *growol* made from the *Lanting* variety and cooked using steaming or a pressure cooker. This result is due to the high resistant starch content in dried-*growol*. The same finding was reported by Figueroa-González et al. (2019) that the growth pattern of probiotic bacteria was almost the same, and *L. rhamnosus* increased from hour zero to hour 15, then growth remained constant. When supplemented with cooked-dried *growol* from M-PC treatment, bacterial growth at the 24th hour tended to be lower than at the 0 hours. The growth pattern of probiotic bacteria depends on the type, and for *L. plantarum* bacteria, peak growth occurs at the 15th hour and then declines (Hidayati, 2010).

Prebiotic index

Tan et al. (2024) studied the potential of banana peel prebiotics on the growth of probiotic *Lactobacillus* spp. *in vitro* in the form of *Berangan* banana peel flour substituted in biscuits as much as 10 to 30% and incubated under aerobic conditions at 37 °C for 24 hours, compared to glucose and inulin, each of which was 2.39% w/w. The results showed that the growth rate of *Lactobacillus* spp. in banana peel biscuit media was higher than in glucose and inulin media. This result was attributed to the presence of fructooligosaccharides in the banana peel, which supported higher bacterial growth than inulin. Dried-*growol* is processed by heating it with high pressure and cooling it in a refrigerator to cause retrogradation. Therefore, its resistant starch content is high, as shown in Table 2. This makes dried-*growol* an interesting candidate for further investigation into its prebiotic potential.

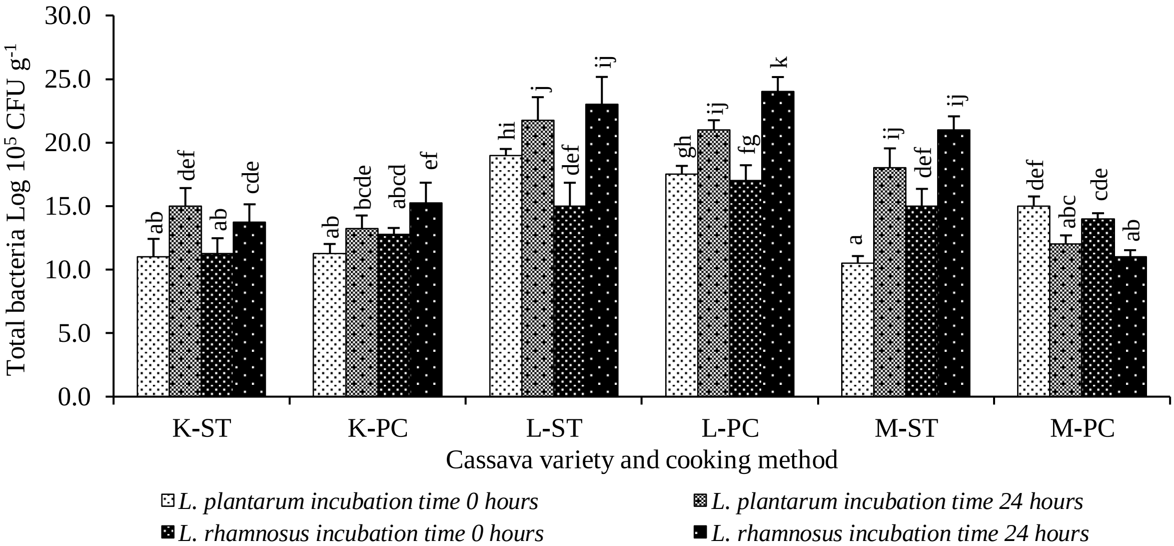

Figure 1. Growth of probiotic bacteria in media plus dried-*growol* at 0 hours (initial) and after 24 hours from M (*Mentega*), L (*Lanting*), K (*Ketan*) varieties and cooked by ST (Steaming) or PC (Pressure cooker)

Note: The different letter on the histogram shows that it is significantly different ($p < 0.05$)

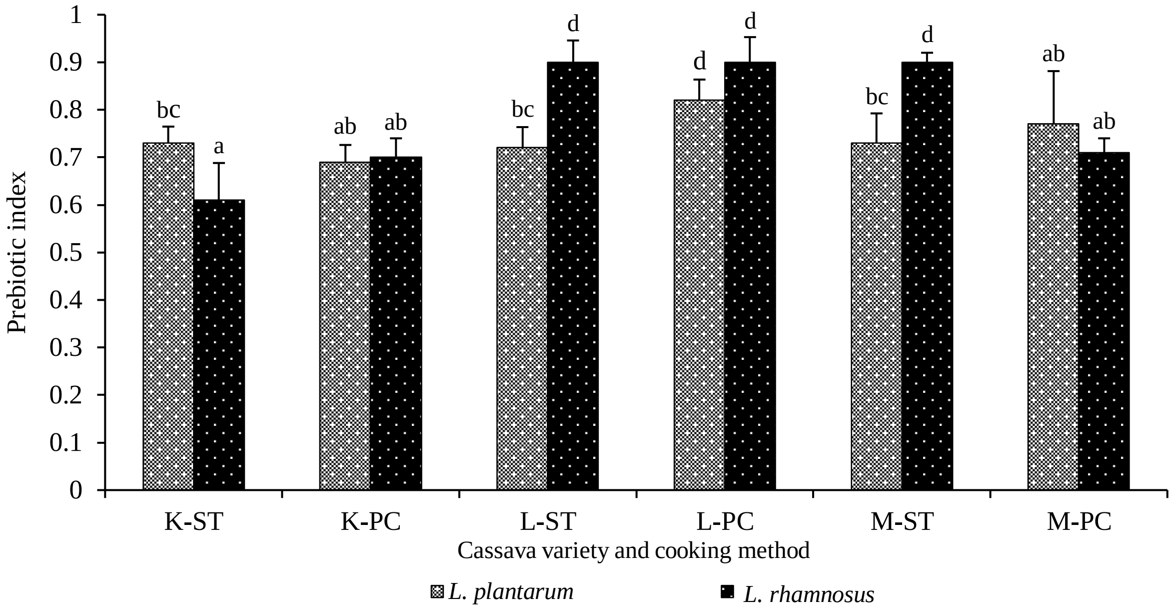

Figure 2. Prebiotic index of dried-*growol* M (*Mentega*), L (*Lanting*), K (*Ketan*) varieties and cooked by ST (Steaming) or PC (Pressure cooker) on probiotic bacteria *L. plantarum* and *L. rhamnosus*

Note: The different letter on the histogram shows that it is significantly different ($p < 0.05$)

The prebiotic index is the ratio of the growth of probiotic bacteria in prebiotics to the growth of probiotic bacteria in carbohydrates (Palframan et al., 2003). The prebiotic used is dried-*growol*, while the carbohydrate, in comparison, is glucose. The prebiotic index is expressed as a maximum number of 1, meaning that the closer it is to 1, the higher the prebiotic properties will be. The prebiotic index of cooked-dried *growol* can be seen in Figure 2. The results of the study show that the prebiotic index of cooked-dried *growol* from cassava varieties *Ketan*, *Lanting*, and *Mentega* cooked using steaming and pressure cooker on the probiotic bacteria *L. plantarum* and *L. rhamnosus* is significantly different.

The probiotic bacteria used in the research were *L. rhamnosus* and *L. plantarum*. Putri et al. (2012) stated that *L. plantarum* and *L. rhamnosus* bacteria predominantly grow in *growol*. The results showed that bacterial growth was high in cooked-dried *growol* from the *Lanting* variety, processed using steaming (L-St) or a pressure cooker (L-PC), and in the *Mentega* variety, processed by steaming (M-St). This is because the *Lanting* variety cooked using steaming or a pressure cooker has a high resistant starch content, whereas in M-St, apart from the resistant starch content, the starch content is also higher. The prebiotic index for *L. plantarum* bacteria is between 0.69 and 0.82, while for *L. rhamnosus*, it is 0.61 and 0.90. The prebiotic index of all dried-*growol* samples was less than 1.0, indicating that the effectiveness as an energy source is lower than glucose. Carbohydrate consumption for each type of probiotic differs so the prebiotic index is also different, for example, *L. rhamnosus* has a high prebiotic index when grown in commercial prebiotic media (Figueroa-González et al., 2019). Putra (2020) stated that the prebiotic index of banana flour, when processed by modifying the boiling process, was 8.8 for *L. plantarum*. In dried-*growol*, even though the prebiotic index is less than 1, it has potential as a prebiotic food because it can be used to grow probiotic bacteria.

CONCLUSIONS

The variety and cooking methods significantly affect the starch and amylose content of dried-*growol*. The starch level and amylose were high in the *Mentega* variety, but the resistant starch levels were the lowest. Dried-*growol*, which is processed from *Lanting* cassava varieties by cooking using steaming or a pressure cooker, contains highly resistant starch and has potential as a prebiotic food as indicated by the increased viability of *L. rhamnosus* and *L. plantarum* bacteria grown in media with dried-*growol* supplements. The dried-*growols* from *Lanting* variety, cooked with steaming and a pressure cooker, contain highly resistant starch between 22.51 and 27.03 g 100 g^{-1} dry matter. Based on its prebiotic index, the dried-*growol Lanting* variety has the potential as a prebiotic functional food with a prebiotic index between 0.82 and 0.90. Dried-*growol* can be used as an alternative staple food, which has excellent health benefits.

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