**International Food Research Journal 29(3): 667 - 675 (June 2022)**
Journal homepage: *http://www.ifrj.upm.edu.my*

# Resistant starch in native *Tacca* (*Tacca leontopetaloides*) starch and its various modified starches

[1]*Nurhayati, R., [2]Suryadi, A. N., [1]Ariani, D., [1]Herawati, E. R. N., [1]Miftakhussolikhah and [2]Marsono, Y.

*[1]Research Division for Natural Product Technology, Indonesian Institute of Sciences, Jl Jogja-Wonosari km 31.5 ds Gading, Playen, Gunungkidul, Yogyakarta, Indonesia*
*[2]Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Gadjah Mada University, Yogyakarta, Indonesia*

**Article history**

*Received:*
*17 October 2020*
*Received in revised form:*
*13 June 2021*
*Accepted:*
*21 November 2021*

**Keywords**

*autoclaving cooling, citric acid hydrolysis, pullulanase, resistant starch, Tacca starch*

**Abstract**

*Tacca* (*Tacca leontopetaloides*) tubers have a high amylose content which can be modified to increase the resistant starch content. The present work therefore aimed to study the influence of acid hydrolysis, enzymes, and autoclaving cooling treatments on resistant starch content formation from *Tacca* starch. To this end, the amylose and resistant starch contents, and the microstructure of the samples were determined. Results showed that *Tacca* starch treated by a combination of acid, enzyme, and autoclaving cooling treatments had the highest resistant starch content which increased from 4.09 to 61.96%, with amylose content increasing from 31.62 to 77.87%. This showed a strong correlation between the increased amylose and resistant starch contents. The microstructure of the starch granules changed from a globular shape into a crystalline structure through SEM observation.

© All Rights Reserved

## Introduction

*Tacca* (*Tacca leontopetaloides*) belongs to the yam family of family Taccaceae, and is native to the tropical Southeast Asia, South Asia, Africa, and Northern Australia. *Tacca* has many names in Indonesia, *i.e., kecondang* (Java), *labin* (Madura), or *jalawure* (Sukabumi). *Tacca* is a type of tuber often found along the coastline of Java and other islands in Indonesia. *Tacca* has the potential to be a source of alternative carbohydrates for arid and coastal areas. *Tacca* becomes the second-largest carbohydrate source after rice, and has been used for a long time to prepare traditional foods in the Garut Regency.

Fresh *Tacca* tubers contain 2 - 3% skin, 6 - 7% fibre, 20 - 30% starch, and 60 - 70% moisture. Dried tubers contain 5.1% protein, 0.2% fat, 89.4% carbohydrates, 2.1% cellulose, 3.2% ash, 0.27% calcium (Ca), 0.2% phosphorus (P), and 2.2% bitter-tasting compounds. *Tacca* tubers also contain compounds such as α-sitosterol, alcohol, tachalin, alkaloids, and steroidal sapogenin (Habila *et al*., 2011). The utilisation of *Tacca* as food is still limited because of the bitter taste. *Tacca* has a high content of starch (83.07 - 89.4%) (Wardah *et al*., 2017), amylose (22.77%), and amylopectin (43.88%). The amylose-amylopectin ratio potentially increases the resistant starch (RS) content. The amylose content (22.27%) can be categorised as medium. Gelatinisation and retrogradation of amylose will increase the RS, while amylopectin can potentially be hydrolysed to produce more amylose content. Gelatinisation, and retrogradation of amylose, and amylopectin hydrolysis are forms of starch modification process. Starch modification for increasing RS is done to increase the functional value of foods.

Resistant starch is a part of the dietary fibres which cannot be digested in the small intestine, but fermented in the large intestine. Resistant starch consists of RS-1 (starch which is physically difficult to digest), RS-2 (granule of RS), RS-3 (retrograded starch), RS-4 (modified starch), and RS-5 (amylose-lipid complex) (Marsono, 2016). Resistant starch is a functional ingredient able to improve human health through its ability to lower blood sugar, stimulate insulin, and prevent colon cancer with the production of short chain fatty acid (SCFA) through fermentation in the large intestine (Zhang and Jin, 2011; Marsono, 2016). Resistant starch also supports the growth of

*Corresponding author.
Email: rifa.lipi@gmail.com
DOI:
https://doi.org/10.47836/ifrj.29.3.18

microflora in the intestinal by increasing the metabolism of cholesterol, and reducing the risk of colon cancer (Ozturk *et al*., 2009). Therefore, the existence of RS in food products needs to be increased.

There are several ways to increase RS in foods. In general, RS is formed due to the retrogradation of amylose and amylopectin, where amylopectin undergoes retrogradation under a special condition (Huang and Rooney, 2001). Marsono (1998) stated that the higher the level of amylose, the higher the resistant starch produced will be. Amylose has a greater recrystallisation capability than amylopectin. It makes a stronger hydrogen bond, and is more difficult to digest. The method usually used for increasing RS content are by starch hydrolysis using acid, enzymes, and physical treatments such as autoclaving cooling. The acid treatment will cause a random hydrolysis on the bonds of α-1,4-glycosidic or α-1,6-glycosidic, which produces shorter amylose chains. The enzymes will hydrolyse specifically α-1,6 of amylopectin glycosidic bond to improve the amylose content. Physical treatment such as autoclaving cooling improves the stability of starch granules against digestive enzyme activity.

Zhang and Jin (2011) reported that the modification of corn starch with pullulanase enzyme increases the RS content to 11.72%. The RS content was influenced by several factors, *i.e.*, the concentration of the enzymes pullulanase added and time of hydrolysis, also, the condition of pH and temperature. Some efforts to increase RS content are autoclaving cooling and acid hydrolysis (Jacobson and Bemiller, 1998; Dupuis *et al*., 2014). Slameut (2015) reported about customising the use of acid, enzymes, and heat moisture treatment (HMT) which showed an increase in RS in the arrowroot starch. Herawati *et al.* (2020) reported an increase in RS content from native *Tacca* starch with autoclaving cooling treatment. No studies on starch modification with a combination of several treatments on *Tacca* starch have been conducted thus far. Therefore, the present work aimed to study the influence of acid hydrolysis, enzymes, and autoclaving cooling treatment on RS content formation from *Tacca* starch.

## Materials and methods

*Tacca starch powder*

*Tacca* tubers were collected from the south coast of Garut District, West Java, Indonesia. The skin was peeled, and the tubers were grounded into pulp. The pulp was filtered using a double-layered thin cloth, and the supernatant was collected. The supernatant was incubated at room temperature (30°C) for 24 h for starch sedimentation. The starch, which settled at the bottom of the container, was separated from the water, and the residue was dried at 50°C in a cabinet dryer till the moisture content became < 4%. *Tacca* starch was sieved on a 100-mesh filter to produce homogenous powder known as native *Tacca* starch (NS). To determine the effects of various modifications, *Tacca* starch was divided into six groups as follows: (1) native starch (NS) was *Tacca* starch without modification, (2) AH was *Tacca* starch hydrolysed by acid, (3) AC was *Tacca* starch modified by autoclaving cooling, (4) AHAC was *Tacca* starch modified by acid and autoclaving cooling, (5) EHAC was *Tacca* starch modified by enzyme and autoclaving cooling, and (6) AHEHAC was *Tacca* starch modified by a combination of acid, enzyme, and autoclaving cooling.

*Acid hydrolysis of Tacca starch*

The acid hydrolysis was performed following the methods described by Hung *et al.* (2016). Briefly, *Tacca* starch was diluted in 0.2 M citric acid solution (10%, w/v), and incubated at 45°C for 24 h. Neutralisation was done with the addition of 1 M NaOH to adjust the pH to 7 to stop the acid hydrolysis. Starch was separated from its supernatant by centrifugation, and then, the residue of starch was dried at 50°C for 48 h. This dry powder starch was labelled AH (acid hydrolysis).

*Autoclaving cooling treatment of Tacca starch*

The autoclaving cooling was performed following the methods described by Zhao and Lin (2009). Briefly, *Tacca* starch was diluted in distilled water (30%, w/v), and gelatinised for 15 min at 65 - 75°C. Gelatinised starch was autoclaved at 121°C for 15 min, and brought to room temperature. The sample was refrigerated (2 - 6°C) for 24 h, removed, and dried in a cabinet dryer at 50°C for 48 h. Dried samples were blended and sieved through a 60-mesh filter. This dry powder starch was labelled as AC (autoclaving cooling) starch.

*Pullulanase hydrolysis of Tacca starch*

The enzyme hydrolysis was performed following the methods described by Zhang *et al*. (2013). Briefly, *Tacca* starch was diluted in distilled

water (30%, w/v), and gelatinised for 15 min at 65 - 75°C. Gelatinised starch was diluted with pullulanase enzyme solution (4 U/grams dry starch) on 0.1 M buffer sodium acetate (pH 5), and incubated at 46°C for 24 h on a water bath shaker. Afterwards, the starch was autoclaved at 121°C for 15 min. The sample was refrigerated (2 - 6°C) for 24 h, removed, and dried at 50°C for 48 h in a cabinet dryer. Dried sample was blended and sieved through a 60-mesh filter. This dry powder starch was labelled as EH (enzyme hydrolysis).

*Resistant starch analysis*

The RS content was analysed following the methods described by Goni *et al*. (1996). Briefly, 25 mg of sample was mixed with 2.5 mL of KCl-HCl buffer at pH 1.5 and 50 µL of pepsin solution (1 g pepsin/10 mL KCl-HCl buffer). The sample was incubated at 40°C for 60 min. Then, 2.25 mL of Trismaleate buffer and 250 µL of α-amylase enzyme solution (40 mg α-amylase/mL Trismaleate buffer) was added. The sample was mixed and incubated at 37°C for 16 h. The sample was then centrifuged for 15 min (3,000 rpm), and the supernatant was decanted. Later, the sample was rinsed with 10 mL of distilled water, and centrifuged (3,000 rpm) again for 15 min before the supernatant was decanted again. To the residue, 3 mL of distilled water and 0.75 mL of 4 M KOH solution were added, mixed, and incubated for 30 min at room temperature. Then, the sample was added with 1,375 mL of 2 M HCl solution, 0.75 mL of sodium acetate buffer at pH 4.75, and 20 µL of the amyloglucosidase enzyme. The sample was mixed and incubated at 60°C for 60 min. The sample was then centrifuged for 15 min (3,000 rpm), and the supernatant was collected in a flask. The sample was rinsed with 10 mL of distilled water, and centrifuged (3,000 rpm) for 15 min. Later, the supernatant was pooled with previous centrifugation, and diluted with distilled water. The sample (0.5 mL) was mixed with 1 mL of glucose assay kit (GOD-PAP) solution, and incubated at 37°C for 30 min. Absorbance of the sample was measured at 500 nm. The RS content was expressed in mg of glucose × 0.9.

*Amylose content*

The amylose content was analysed following the methods described by Juliano (1971). Briefly, 100 mg of sample with 1 mL of 95% ethanol and 9 mL of 1 N NaOH were heated in boiling water until all the ingredients formed a gel, which was then entirely transferred into a flask. The sample was diluted with distilled water until the volume was 100 mL. Next, 5 mL of sample solution was mixed with 1.0 mL of 1 N acetic acid and 2 mL of iodine solution. The mixture was diluted in distilled water until it reached a volume of 100 mL, and was allowed to stand for 20 min. The intensity of the blue colour formed and its absorbance was measured at 625 nm.

*Moisture content*

The moisture content was analysed using the gravimetric method (AOAC, 1984). Briefly, 2 g of the sample were dried at 105°C until a constant weight was obtained. The weight reduction was taken as the amount of water in the sample. Moisture content is the percentage of the amount of water which evaporates or is lost from the sample.

*Starch microstructure*

Scanning electron microscopy (SEM) was carried out on Hitachi SU 3500. The modified starch sample was sputtered with gold for 10 min at 10 mA. The sample was observed and photographed using 3.00 kV accelerating potential.

*Statistical analysis*

The IBM SPSS Statistics 23 software was used for statistical analysis to determine whether there was a significant difference or effect of each modified treatment on the levels of RS and amylose samples, with a significance value (α) of 0.05. The ANOVA method was used for the analysis, and if significant differences were discovered, the analysis was continued with Duncan's *post hoc* test.

## Results and discussion

*The effect of Tacca starch modifications on the RS content*

The RS contents in *Tacca* starch treated with autoclaving cooling and acid treatment increased as compared to NS from 4.09% to 7.88% (AH), and to 17.82% (AC) (Figure 1). RS content of samples increased with a combination of treatments between acid, enzymes, and autoclaving cooling. Samples with a combination of acid and autoclaving cooling treatment increased RS content to 44.61% (AHAC), and samples with a combination of enzymes and autoclaving cooling increased to 54.68% (EHAC). The combination between acid, enzymes, and autoclaving cooling yielded the highest RS content of

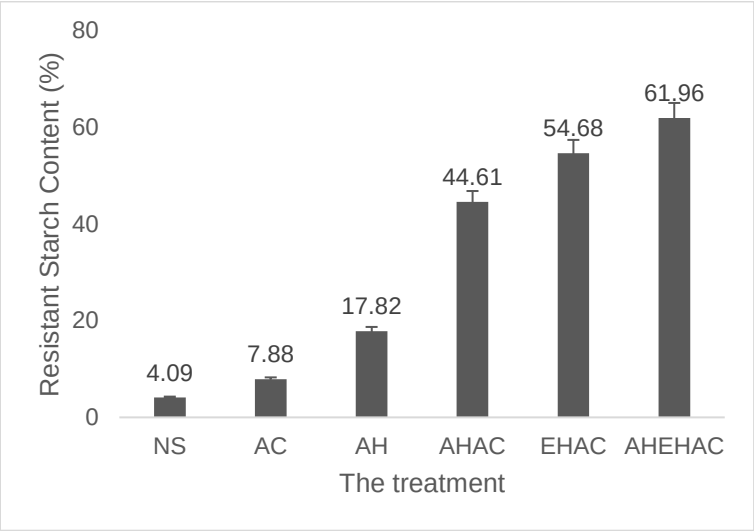


**Figure 1.** Resistant starch contents (%) of native and various modified *Tacca* starches before and after treatments. NS = native starch; AH = acid hydrolysis; AC = autoclaving cooling; AHAC = acid hydrolysis and autoclaving cooling; EHAC = enzyme hydrolysis and autoclaving cooling; and AHEHAC = acid hydrolysis, enzyme, and autoclaving cooling.

61.96% (AHEHAC). During acid treatment, random hydrolysis occurs predominantly on α-1,4-glycosidic bond, and slightly on α-1,6-glycosidic bond to produce more amylose (Lehmann and Robin, 2007). Combination with autoclaving cooling increased the RS content on samples. Autoclaving cooling treatment improved the capability of starch retrogradation especially at cooling stage (Liu, 2005). According to Milasinovic *et al*. (2010), warming the starch above 100°C, and autoclaving cooling increased the formation of RS, which makes it difficult to digest. During the autoclaving cooling process, amylose will bond with other amyloses through hydrogen bonds that form a double helix structure. Then, it will be joined with other double helix structures, thus forming crystals that are resistant to digestive enzymes (Shin *et al*., 2004; Mutungi *et al*., 2009). Setiarto *et al.* (2018) also reported the increase in RS in taro flour by autoclaving cooling treatment to 7.92% from its native (4.13%). The present work demonstrated that a combination of acid hydrolysis and autoclaving cooling treatment resulted in a higher RS than the acid-only treatment, namely from 4.09% (NS) to 44.61% (AHAC), which was higher than 7.88% (AH). According to Zhao and Lin (2009), hydrolysis treatment on corn starch with citric acid of 0.1 M for 24 h followed by autoclaving cooling increased the RS content to 8 - 11% from NS.

Enzymatic hydrolysis of starch with pullulanase enzyme specifically breaks α-1,6-glycosidic bond, thus resulting in amylose and increasing the level of RS (Zhang and Jin, 2011). A combination between enzymatic hydrolysis and autoclaving cooling increased the RS content to 54.68%. Mutungi *et al*. (2009) hydrolysed the cassava starch with pullulanase enzyme (25 U/g starch) for 24 h, then applied the autoclaving cooling at 121°C for 15 min. Its modification result increased the level of RS from 21.4 to 88.4%.

The highest RS content of *Tacca* starch (61.96%) was obtained from the combination of acid hydrolysis, enzymes, and autoclaving cooling (AHEHAC). This result is similar to a report by Slameut (2015) which found that the highest RS (36.3%) in the treatment was with acid, pullulanase enzyme, and autoclaving cooling. The difference in RS levels was caused by the difference in the acid used or the difference in the amylose level of the NS. In the present work, citric acid was used to hydrolyse *Tacca* starch, which allowed for the formation of RS-5, namely amylose-lipid complex, whereas in previous research, hydrochloric acid was used to hydrolyse the corn starch without forming an amylose complex.

*The relationship between RS level with amylose level*

Increased level in RS was influenced and closely related to the amylose level (Figure 2). Methylated amylose will form RS because of the nature and tendency of the amylose to re-join, which is why the amylose content becomes a major factor in

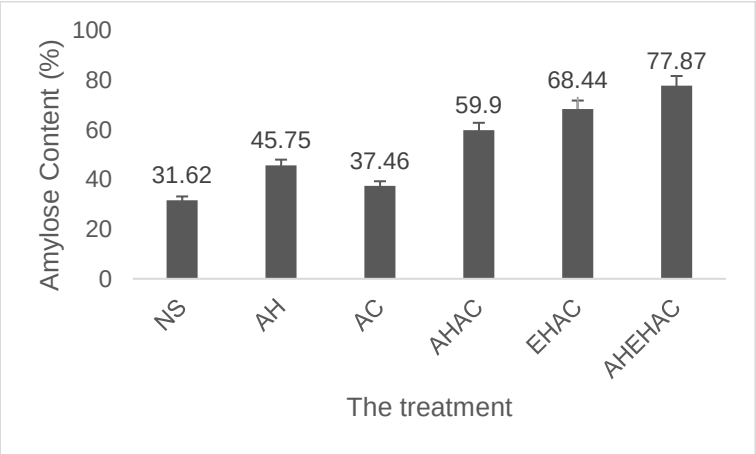


**Figure 2.** Amylose content (db) of native and various modified *Tacca* starches before and after treatments. NS = native starch; AH = acid hydrolysis; AC = autoclaving cooling; AHAC = acid hydrolysis and autoclaving cooling; EHAC = enzyme hydrolysis and autoclaving cooling; and AHEHAC = acid hydrolysis, enzyme, and autoclaving cooling.

the formation of RS (Luckett and Wang, 2016). During starch retrogradation, amylose will reform a compact structure in the presence of hydrogen bonds (Sajilata *et al.*, 2006). The hydrolysis process with acids and enzymes carried out before the autoclaving cooling process aimed to de-branch the α-1,4-glycosidic and α-1,6-glycosidic bonds which produce shorter glucan chains, so that the amylose content in the analysis became more numerous. The autoclaving cooling (AC) process also has a role in the hydrolysis of starch. An increase in amylose content in the autoclaving cooling treatment occurs due to the degradation of long- to short-chain amylose, which results in an increase of amylose content (Shin *et al.*, 2004). In the present work, the obtained amylose contents in NS, AC, and AH treatments were 31.62, 37.45, and 45.73%, respectively. The amylose level increased to 59.9% with the combination of acid treatment and autoclaving cooling (AHAC), whereas enzyme treatment with autoclaving cooling (EHAC) increased the amylose level to 68.42%. Finally, the highest amylose level was found in samples with a combination of acid treatment, enzymes, and autoclaving cooling (AHEHAC) at 77.87%.

From the results of RS and amylose contents obtained, it can be seen that the RS content was strongly positively correlated with amylose content with an $R^2$ value of 0.8974 (Figure 3).

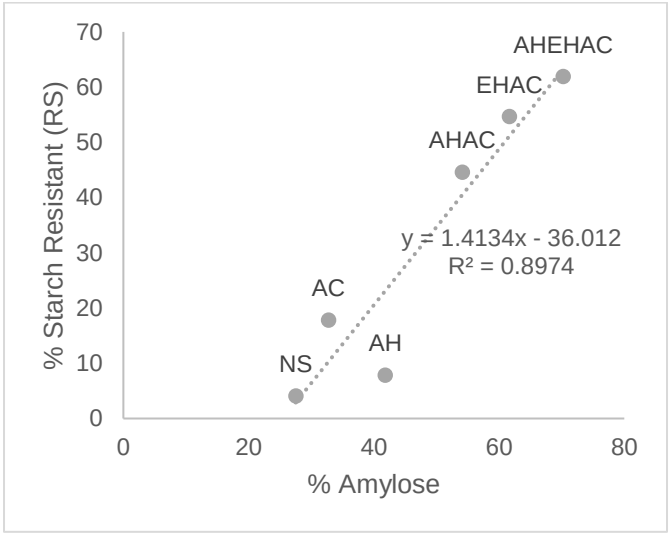


**Figure 3.** Correlation between resistant starch contents and amylose contents of native and various modified *Tacca* starches before and after treatments. NS = native starch; AH = acid hydrolysis; AC = autoclaving cooling; AHAC = acid hydrolysis and autoclaving cooling; EHAC = enzyme hydrolysis and autoclaving cooling; and AHEHAC = acid hydrolysis, enzyme, and autoclaving cooling.

This proves the recrystallisation of amylose, which forms a compact structure through hydrogen bonds to form starch that is resistant to digestive enzymes (Sajilata *et al*., 2006). According to Sarwono (2006), the correlation value between 0.75 and 0.99 shows a very strong correlation between the amylose and RS level. In AHAC, the relationship between RS content and amylose content was not linear. The increase in RS in AH can be caused by the esterification between organic acid molecules and starch, thus causing starch to become resistant with digestive enzymes (Hung *et al*., 2016). However, the RS value was much lower than that in samples with autoclaving cooling treatment due to the absence of heating and cooling treatments, which causes starch retrogradation and recrystallisation of amylose structures.

Based on statistical analyses, it was concluded that amylose content in each treatment had a significant difference with natural *Tacca* starch (NS) and other treatments. Each modification treatment yielded a significant increase in the amylose content. This indicated that the real effect of debranching on α-1,4-glycosidic and α-1,6-glycosidic bonds in starch was due to the acid hydrolysis and enzymes. It can also be concluded that the RS level of each treatment had a significant difference with NS, except for samples with acid treatment. This could be due to the fact that acid treatment does not cause gelatinisation and retrogradation on the starch, which means that the amylose formed from the hydrolysis does not undergo recrystallisation to form a more compact structure. In addition, the statistical results of each treatment also showed a significant difference between them. This indicated that the increase in RS level could likely be due to the formation of new amylose crystals that are resistant to digestive enzymes. The chemical properties of the samples are shown in Table 1.

**Table 1.** Chemical properties of native and various modified *Tacca* starches before and after treatments.

| Treatment | Moisture (% wb) | Amylose (% db) | Resistant starch (% db) |
|---|---|---|---|
| Native starch (NS) | 12.96 ± 0.014 | 31.62 ± 0.052$^{a}$ | 4.09 ± 0.003$^{a}$ |
| Acid hydrolysis (AH) | 8.58 ± 0.024 | 45.73 ± 0.010$^{b}$ | 7.88 ± 0.011$^{a}$ |
| Autoclaving cooling (AC) | 12.56 ± 0.002 | 37.45 ± 0.010$^{c}$ | 17.82 ± 0.029$^{b}$ |
| Acid hydrolysis and autoclaving cooling (AHAC) | 9.69 ± 0.002 | 59.90 ± 0.008$^{d}$ | 44.61 ± 0.050$^{c}$ |
| Enzyme hydrolysis and autoclaving cooling (EHAC) | 9.94 ± 0.009 | 68.42 ± 0.016$^{e}$ | 54.68 ± 0.046$^{d}$ |
| Acid hydrolysis, enzyme, and autoclaving cooling (AHEHAC) | 9.81 ± 0.007 | 77.87 ± 0.016$^{f}$ | 61.96 ± 0.028$^{e}$ |

Values are mean ± standard error. Means followed by different lowercase superscripts in the same column are significantly different ($p < 0.05$).

*The microstructure of native starch and its modifications*

From SEM analyses, it could be seen that the physical nature of the starch granule crystals was formed as a result of the modification (Figure 4). In NS, the surface of the granule structure was smooth and round. The size of starch granules also varied with the size of the largest starch granules observed at 1,000× magnification was approximately 24 µm. In acid hydrolysis treatment, holes visible on the surface of starch granules indicated starch granule debranching, and also the release of amylose and amylopectin molecules from starch granules. It also indicated the presence of starch granules that began to accumulate, thus proving the occurrence of cross bonds between organic citric acid and starch molecules (Zhou *et al*., 2016).

In samples with other treatments, visible changes were present on the shape of the starch granule molecules, which turned into a rough surface with a shape that resembled a crystal. According to Miao *et al*. (2011), starches that have undergone gelatinisation will change the shape and nature of the native starch granules, and recombine with other molecules when retrogradation occurs. This causes the formation of granule shapes which resemble crystals. The better retrogradation ability will cause granule crystals to have a larger size, as seen in samples treated with acids, enzymes, and autoclaving cooling (AHEHAC) which showed a larger size than other samples. The physical properties of starch granules are also related to the RS content, as the samples with rough crystalline shapes and larger sizes generally indicated a higher RS content.

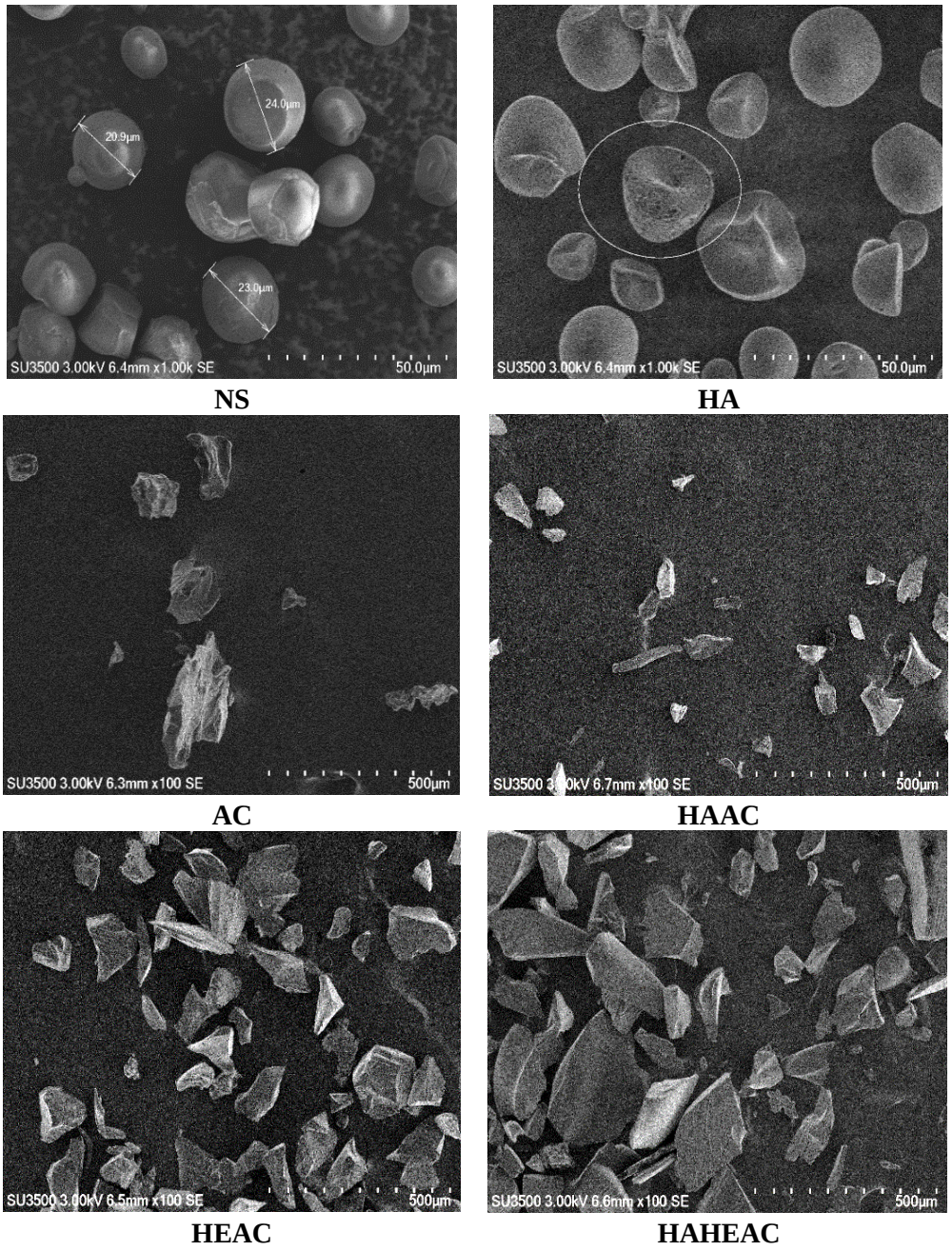


**Figure 4.** SEM photograph of native and various modified *Tacca* starches before and after treatments. NS = native starch; AH = acid hydrolysis; AC = autoclaving cooling; AHAC = acid hydrolysis and autoclaving cooling; EHAC = enzyme hydrolysis and autoclaving cooling; and AHEHAC = acid hydrolysis, enzyme, and autoclaving cooling.

In acid treatment, random hydrolysis occurs and predominantly breaks the α-1,4-glycosidic bond and a few α-1,6-glycosidic bonds that connect the amylose chain to produce shorter and more amylose chains (Lehmann and Robin, 2007). The combination with autoclaving cooling treatment could increase the RS level because it could increase the ability of starch retrogradation, and then cooling process will occur more quickly (Liu, 2005). According to Milasinovic *et al*. (2010), heating starch above 100°C can also increase the formation of RS, and treatment of heating and storage (cooling) can reduce the digestibility of starch due to the increased level in RS. During the autoclaving cooling process, there is a rearrangement of starch molecules between amylose and amylopectin so that the hydrogen bonds in starch will become stronger (Shin *et al*., 2004). Amylose will bind to other amylose through hydrogen bonds to form a double helix structure, which will then join with other double helix structures, thus forming

crystals that are resistant to digestive enzymes. This is in accordance with the present work where the RS level in samples with a combination of acid hydrolysis and autoclaving cooling treatments increased higher than those using acid alone, namely from 4.09% (NS) to 44.61% (AHAC) as compared to 7.88% (AH). According to Zhao and Lin (2009), corn starch which was treated with 0.1 M citric acid for 24 h and followed by an autoclaving cooling could also increase the RS level by 8 - 11%. Enzyme hydrolysis using pullulanase is different from hydrolysis using acids, where there is a specific debranching that breaks the α-1,6-glycosidic bonds to produce amylose, and increases the RS level (Zhang and Jin, 2011). The amylose content which increases from the debranching of α-1,4-glycosidic and α-1,6-glycosidic bonds will increase the retrogradation ability of starch. Increased level of RS also occurred in the treatment of enzymes combined with autoclaving cooling which increased to 54.68%. This is consistent with the research of Mutungi *et al*. (2009) which conducted the debranching process of cassava starch with pullulanase enzyme (25 U/gram starch) for 24 h and autoclaving cooling process at 121°C for 15 min, and showed significant increase in RS level from 21.4 to 88.4%.

The highest RS level was found in the combination of acid, enzymes, and autoclaving cooling treatment, which was 61.96%. A similar result was also reported by Slameut (2015) who showed that the highest RS level (36.3%) was found in treatment with acid, pullulanase enzymes, and autoclaving cooling (AHEHAC) as compared to treatment with autoclaving cooling (AC), acid (AH), acid and autoclaving cooling (AHAC), and treatment with enzymes and autoclaving cooling (EHAC). This might be due to differences in the acid used and differences in RS levels in NS. In the present work, citric acid was used to hydrolyse *Tacca* starch, which enabled the formation of RS-5, which is amylose-lipid complex; whereas in previous studies, HCl acid was used and only aimed to hydrolyse starch without being able to form complex with amylose.

## Conclusion

Modification of *Tacca* starch could increase the amylose and resistant starch contents, with the highest contents (RS = 61.96%; amylose = 77.87%) were obtained by a combination of acid hydrolysis, enzymes, and autoclaving cooling. An increase in amylose content was also followed by an increase in resistant starch content. Amylose had a strong correlation with resistant starch formed with the aforementioned modifications. The native starch microstructure had a round or oval shape and a smooth surface, while the modified starch had a crystal shape, and was of a larger size than the native starch.

## Acknowledgement

We convey our thanks to the *Riset Unggulan* program of the Indonesian Institute of Sciences for financially supporting the present work.

## References

Association of Analytical Chemist (AOAC). 1984. Official methods of analysis of the AOAC international. United States: AOAC.

Dupuis, J. H., Liu, Q. and Yada, R. Y. 2014. Methodologies for increasing the resistant starch content of food starches: a review. Comprehensive Reviews in Food Science and Food Safety 13(6): 1219-1234.

Goni, I., Garcia-Diz, L., Manas, E. and Saura-Calixto, F. 1996. Analysis of resistant starch: a method for foods and food products. Food Chemistry 56: 445-449.

Habila, J. D., Bello, I. A., Dzikwe, A. A., Ladan, Z. and Sabiu, M. 2011. Comparative valuation of phytochemicals, antioxidant and antimicrobial activity of four medicinal plants native to Northern Nigeria. Australian Journal of Basic and Applied Sciences 5(5): 537-543.

Herawati, E. R. N., Ariani, D., Na'imah, H., Nurhayati, R., Miftakhussolikhah and Marsono, Y. 2020. Effect of autoclaving cooling treatments on chemical characteristic and structure of *Tacca* (*Tacca Leontopetaloides*) starch. In Proceeding of the 5th International Conference on Food, Agriculture and Natural Resources (FANRes 2019), p. 169-172. Indonesia.

Huang, D. P. and Rooney, L. W. 2001. Starches for snacks foods. United States: CRC Press.

Hung, P. V., Vien, N. L. and Lan-Phi, N. T. 2016. Resistant starch improvement of rice starches under a combination of acid and heat-moisture treatments. Food Chemistry 191: 67-73.

Jacobson, M. R. and Bemiller, J. N. 1998. Method for determining the rate and extent of accelerated starch retrogradation. Cereal Chemistry 75(1): 22-29.

Juliano, B. O. 1971. Simplified assay for milled rice amylose. Cereal Science Today 6: 334-340.

Lehmann, U. and Robin, F. 2007. Slowly digestible starch – its structure and health implication: a review. Trends in Food Science and Technology 18: 346-355.

Liu, Q. 2005. Understanding starches and their role in foods. In Cui, S. W. (ed). Food Carbohydrates - Chemistry, Physical Properties, and Applications, p. 309-356. United States: CRC Press.

Luckett, C. R. and Wang, Y. J. 2016. Effects of β-amylolysis on the resistant starch formation of debranched corn starches. Journal of Agricultural and Food Chemistry 60(18): 4751-4757.

Marsono, Y. 1998. Formation of resistant starch (RS) and chemical composition of some carbohydrate-rich foodstuffs in processing. agriTECH 19(3): 124-127.

Marsono, Y. 2016. The role and mechanism of resistant starch (RS) in reducing plasma glucose concentration. In Proceeding of the International Food Conference 2016 - Innovation of Food Technology to Improve Security and Health. Indonesia: Widya Mandala Catholic University.

Miao, M., Jiang, B. and Zhang, T. 2011. Effect of pullulanase debranching and recrystallization on structure and digestibility of waxy maize starch. Carbohydrate Polymers 76: 214-221.

Milasinovic, M. S., Milica, M. R. and Ljubica, P. D. 2010. Effect of autoclaving cooling and pullulanase debranching on the resistant starch yield of normal maize starch. Journal of the Serbian Chemical Society 75(4): 449-458.

Mutungi, C., Rosta, F., Onyangob, C., Jarosa, D. and Rohma, H. 2009. Crystallinity, thermal and morphological characteristics of resistant starch type III produced by hydrothermal treatment of debranched cassava starch. Starch 61: 1-12.

Ozturk, S., Koksel, H. and Perry, K. W. N. 2009. Characterization of resistant starch samples prepared from two high-amylose maize starches through debranching and heat treatments. Cereal Chemistry 86(5): 503-510.

Sajilata, M. G., Singhal, R. S. and Kulkarni, P. R. 2006. Resistant starch – a review. Comprehensive Reviews in Food Science and Food Safety 5(1): 1-17.

Sarwono. 2006. Quantitative and qualitative research methods. Yogyakarta: Graha Ilmu.

Setiarto, R. H. B., Jenie, B. S. L., Faridah, D. N., Saskiawan, I. and Sulistiani. 2018. Effect of lactic acid bacteria fermentation and autoclaving cooling for resistant starch and prebiotic properties of modified taro flour. International Food Research Journal 25(4): 1691-1697.

Shin, S., Byun, J., Park, K. W. and Moon, T. W. 2004. Effect of partial acid and heat moisture treatment of formation of resistant tuber starch. Cereal Chemistry 81(2): 194-198.

Slameut, F. 2015. Modification of arrowroot starch (*Marantha arundinaceae* L.) with acid, enzymes, and physical treatment for producing type 3 resistant starch (RS 3). Indonesia: IPB University, MSc thesis.

Wardah, Sambas, E. N., Ridwan and Ariani, D. 2017. Starch product of wild plants species jalawure (*Tacca leontopetaloides* L.) Kuntze as the source of food security in the south coastal west Java. IOP Conference Series: Materials Science and Engineering 193: article ID 012035.

Zhang, H. and Jin, Z. 2011. Preparation of resistant starch by hydrolysis of maize starch with pullulanase. Carbohydrate Polymers 83: 865-867.

Zhang, H., Tian, Y., Bai, Y., Xu, X. and Jin, Z. 2013. Structure and properties of maize starch processed with a combination of α-amylase and pullulanase. International Journal of Biological Macromolecules 52: 38-44.

Zhao, X. H. and Lin, Y. 2009. The impact of coupled acid or pullulanase debranching on the formation of resistant starch from maize starch with autoclaving cooling cycles. European Food Research and Technology 23: 179-184.

Zhou, J., Tong, J., Xingguang, S. and Ren, L. 2016. Hydrophobic starch nanocrystals preparations through crosslinking modification using citric acid. International Journal of Biological Macromolecules 91: 1186-1193.