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# Effect of Processing Conditions on Production of Resistant Starch Type III (RS3) from Breadfruit Starch

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## *ABSTRACT*

Resistant starch type III (RS3) possess various beneficial physiological effects as well as functionality in food containing resistant starch (RS). In this study, RS3 is produced from breadfruit (*Artocarpus altilis*) starch using different processing conditions involving disruption of starch granules, enzymatic debranching of starch polymer, starch retrogradation and drying. Then, the morphology and size of the starch granules were observed using scanning electron microscope (SEM). A sample with the highest RS content (54.59%), denoted as P8 was produced when the breadfruit starch was suspended in distilled water, gelatinised by heating at 90°C for 30 minutes, followed by starch debranching with 20 New Pullulanase Unit Novo (NPUN) enzyme per g starch at 60°C for 24 hours. The suspension was then autoclaved at 121°C for 1 hour and cold stored at 4°C for 24 hours. Further treatment of P8 sample with 0.5 M HCl acid at 60°C for 24 hours, produced HCl-breadfruit RS3 with 57.86% of RS content. It was observed that hydrolysis of breadfruit RS3 sample with HCl produced smoother starch granules with more crystalline region and proportionally increased the RS content when compared to other treated sample. This study revealed that different processing conditions were significantly influenced the percentage of yield and properties of RS type III produced from breadfruit starch.

**Keywords:** Resistant starch type III, *Artocarpus altilis*, breadfruit starch, pullulanase, granules morphology

## INTRODUCTION

Resistant starch (RS) is referred as non-digestible starch fraction that resists absorption and digestion along the gastrointestinal tract and may be completely or partially fermented in the colon (Zi-Ni et al., 2015). Resistant starch type III (RS3) is widely studied among other types of RS because of its beneficial functionality in food applications such as thermally stable, high pasting temperature, improved texture, appearance and organoleptic properties (Demirkesen-Bicak et al., 2018). Interestingly, its thermal stability helps foods with RS3 addition to retain their functional benefits even after cooking (Zi-Ni et al., 2015). It has therefore made RS3 preferable as

a functional food ingredient amongst other RS. In addition, in food processing that involves heat and humidity, RS1 and RS2 have been destroyed in most cases but may form RS3 (Fuentes-Zaragoza et al., 2010). In a study by Öztürk and Mutlu (2018), the health benefits of RS3 were thoroughly clarified such as increased satiety, improved bowel health, glycemic and insulinemic responses, and reduced energy intake.

Most of processed foods generally contain appreciable amount of RS and it depends on the type of the starch, type of processing, the duration and storage conditions (Leong et al., 2007). RS3 is a type of retrograded non-granular starch which formed during cooling of cooked starch (Fuentes-Zaragoza et al., 2011). Generally, many studies have been conducted to produce and increase the amount of RS in foods using different starch modification methods such as genetic methods, physical and enzymatic treatments, chemical modifications and other methods as reviewed by Dupuis et al. (2014). However, it involves combination of processing techniques and retrogradation of the starch specifically for the production of RS3 as reported by Gao et al. (2011). In addition, RS3 can also be produced by food processing methods that lead to changes in both starch structure and starch resistibility against digestion (Ma et al., 2018).

A previous study conducted by Nor et al. (2014) had produced healthier fish cracker fortified with sago RS, which was modified from sago starch using different processing methods. This modified sago starch showed may increase the starch resistance towards digestive enzymes. Moreover, a study done by Zi-Ni et al. (2015) has detailed out examples of physical and enzymatic treatments that comprised of four sequential processing steps including the disruption of starch granules (starch gelatinisation), enzymatic debranching of starch polymer with debranching enzyme (pullulanase), starch retrogradation process and drying for the production of RS3.

Previous researches have focused on the production of RS3 from readily accessible starch sources for instance maize (Ai, 2013), wheat, rice and potato (Dhita et al., 2009), but less researches recorded on the production of RS3 from tropical fruits' starch such as breadfruit starch. Breadfruit is known as underutilised food crop in Malaysia and diminutive information was reported on its nutritional values and functional properties. Therefore, it is noteworthy to explore its potential use in food applications (Nwokocha & Williams, 2011).

Thus, this study aims to determine effect of (8) processing conditions for the production of RS3, purify the RS3 using acid hydrolysis method and to determine the morphology of RS3 produced from the breadfruit starch using scanning electron microscope (SEM). It is hoped that this present study will provide an important guidance in producing RS3 from the breadfruit starch by using pre-studied optimal conditions from several treatments.

## MATERIALS AND METHODS

### Materials

Breadfruit flour was produced by processing the fresh breadfruits where it involved the skin peeling, skin removing, pulp washing, slicing, drying and grinding of the dried breadfruits. The starch of breadfruit was extracted according to Madzlan et al. (2012) and was used for the production of breadfruit RS type III (breadfruit RS3). Eight (8) different processing conditions were conducted by four sequential steps that were disruption of starch granules, enzymatic debranching of starch polymer, starch retrogradation and drying. A technical grade debranching enzyme, pullulanase microbial (Promozyme® D2) was purchased from Sigma Aldrich (Germany) with a specific activity of ≥1000 NPUN/g (New Pullulanase Unit Novo) pullulanase and a density of 1.10 - 1.20 g/mL. Pullulanase enzyme is a type of glucanase that degrades pullulan. One (1) NPUN is defined as the amount of enzyme, which under standard conditions hydrolyzes pullulan, liberating reducing sugar with reducing power equivalent to 1 μmole glucose per minute. Other enzymes, pepsin (RM712), α-amylase (A0447) and amyloglucosidase (A1602) were purchased from HiMedia Laboratories (Mumbai, India), Tokyo Chemical Industry (Tokyo, Japan) and Sigma Aldrich (Steinheim, Germany) respectively. Other chemicals used in this experiment were analytical grade purchased from Merck Milipore (Germany), Bendosen Laboratory Chemicals

(Norway), Nacalai Tesque, Inc. (Kyoto, Japan), Tokyo Chemical Industry (Tokyo, Japan), Sigma, Aldrich (Steinheim, Germany) and HiMedia Laboratories Pvt. Ltd. (Mumbai, India).

**Production of breadfruit resistant starch Type III (breadfruit RS3)**

The production of breadfruit RS3 in this study was adapted from Zi-Ni et al. (2015). In every process, factors that were thought to influence the amount of the RS3 produced were determined for RS content. The experiments were conducted in triplicates. Fig. 1 summarises the flow of experiment and processing conditions. There were four main processing steps involved in the production of breadfruit RS3:

*Step 1: Disruption of the starch granules (gelatinisation by heat treatment)*

Twenty (20) gram of breadfruit starch (20%, w/v) was suspended in either 100 mL of 0.1 M acetate buffer, pH 5 or distilled water. Then starch slurry was subjected to heat treatment, either boiling in water bath (90°C) for 30 minutes (until gelatinised) and/or autoclaving (Autoclave Model SX-700 Tomy Digital Biology, Japan) at 121°C for 1 hour. The starch gel was then cooled to 60°C prior to the enzymatic debranching step (Step 2).

*Step 2: Enzymatic debranching*

The debranching pullulanase enzyme (20 NPUN/g) was added into cooled gelatinised starch samples and the starch-enzyme suspension was incubated in water bath shaker (Memmert GmbH & Co. KG, Germany) at 60°C for 24 or 48 hours separate incubation times. After incubation was finished, the reaction was inactivated by heating the starch-enzyme suspension in a water bath at 80°C for 15 minutes.

*Step 3: Thermal processing and cold storage to induce retrogradation*

Starch-enzyme suspensions from Step 2 undergone thermal processing by autoclaving at 121°C (0.1 Mpa) for 1 hour and was cooled to ambient temperature (25°C) before being stored for 24 hours in temperature of 4 °C for cold storage process. Starch-enzyme suspensions were subjected to either one or two cycles of thermal and cold storage processing in order to increase RS content in the samples.

*Step 4: Drying and grinding*

The breadfruit RS3 samples were oven-dried at 50°C until its moisture content was less than 13% as previously mentioned by Appiah and Oduro (2011) and was ground into fine powder with particles size of 125 μm (120 Mesh size). Each processing conditions was tested sequentially according to the steps in RS3 production, leading to the formation of eight (8) different processing conditions. All breadfruit RS3 samples were denoted as sample P1 (processing 1) until sample P8 (processing 8).

**Acid hydrolysis of breadfruit RS3 to produce HCl-breadfruit RS3**

Breadfruit RS3 samples with the highest RS content were further subjected to acid hydrolysis by following Zi-Ni et al. (2015) method with some modifications. The samples were treated with 0.5 M HCl at a ratio of 1: 3.5 (breadfruit RS3 : HCl) at 60°C for 24 hours with continuous shaking (150 rpm) using orbital shaker (Model SI-600, Lab Companion, South Korea) to produce HCl-breadfruit RS3. The starch slurry was centrifuged at 3610 rpm for 15 minutes using centrifuge (Model 2-16 Sartorius, Gòttingen, Germany). Then the starch pellet was washed with distilled water several times until the supernatant turned colourless and subject to drying in oven (Memmert GmbH & Co. KG, Germany) at 50°C until its moisture content was less than 13%. Dried starch was then ground into fine particles size (125 μm /120 Mesh size).

**Determination of Resistant Starch Content of breadfruit RS3 and HCl-breadfruit RS3**

The total RS content of breadfruit RS3 and HCl-breadfruit RS3 samples were determined according to Goni et al. (1996) with modifications. Both samples (50 mg, dry basis) were weighed in a 50 mL centrifuge tube and protein removal from samples were done using pepsin enzyme solution (400 units/mg sample) (pH 1.5) at 40°C for 1 hour. The samples were left to cool at ambient temperature (25°C) before addition of 4.5 mL of 0.1 M TRIS-meleate buffer (pH 6.9). Then, the pH of solution was adjusted to 6.9 with 0.5 M NaOH. Next, the digestible starch of samples were hydrolysed with α-amylase enzyme solution (4 units/mg sample) at 37°C for 16 hours with constant shaking (150 rpm). Then, precipitated samples were solubilised with 4.0 M potassium hydroxide (KOH) and were hydrolysed with amyloglucosidase enzyme solution (0.12 U/mg sample) at 55°C for 45 minutes with a constant shaking (150 rpm). Finally, the RS content of samples were determined by the calculation of liberated glucose with conversion factor, 0.9 of sample and the formula was given as supplied by the protocol of GOPOD reagent (Robonik, India) as follows:

Glucose Content of sample (mg glucose) = (S/A) × B **Eqn. 1**
S: Concentration of standard glucose used (mg/mL)
A: Absorbance of standard glucose
B: Absorbance of sample

Total Resistant Starch Content (%) = (mg glucose × 0.9) × 100 **Eqn. 2**

**Observations of Granules morphology**

Granules morphology of selected breadfruit RS3 with the highest RS content and HCl-breadfruit RS3 samples were observed using scanning electron microscope (SEM) Model JEOL JSM- 6360LA (Jeol Ltd, United States). The samples were viewed under 300× and 500× magnification as followed to the method by Nwokocha and Williams (2011) with slightly modifications. Initially, the samples were evenly tapped on double adhesive tape tucked to a circular aluminium stubs. The circular aluminium stub with samples on it was placed in vacuum chamber of autofine coater machine, model JFC-1600 (Jeol Ltd, United States) and gold coating process was carried out for 140 seconds. The stub with gold coated sample was then placed in the SEM chamber which was evacuated before the electron beam was turned on. The SEM was operated at 10kV/2.05A and the aperture size was fixed at 3.

**Statistical Analysis**

The Statistical Package for Social Science, Version 24.0 (IBM SPSS Inc., USA) was used for the data analysis. All the analyses were run in triplicate and the results were expressed as mean ± SD (standard deviation). The differences between samples means were statistically computed using one-way analysis of variance (ANOVA). The means comparisons were computed using Duncan post-tests for RS content of eight (8) samples breadfruit RS3. Independent *t* test was used to analyse between RS content of breadfruit RS3 and HCl-breadfruit RS3 samples. The level of significance was set at $p < 0.05$ (95% confidence level).

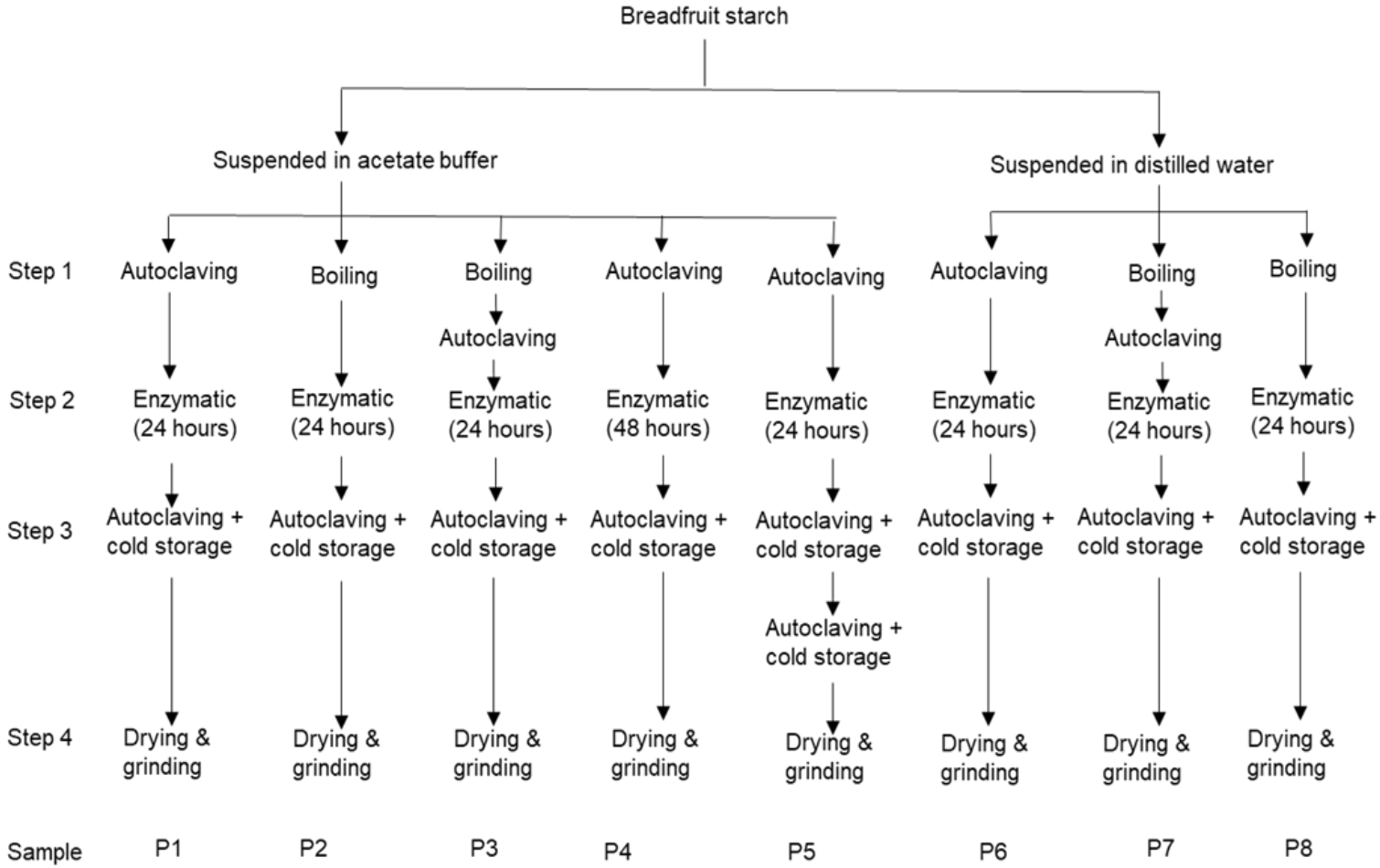


**Fig. 1.** Different processing conditions for the production of breadfruit RS3

## RESULTS AND DISCUSSION

### Resistant Starch Content of breadfruit RS3 and HCl- breadfruit RS3

*Breadfruit RS3*

Table 1 shows the RS content of breadfruit RS3 samples obtained from eight different processing conditions (P1-P8). A significant decreasing order for RS content in samples at $p< 0.05$ was obtained and the range starts from 54.59% to 28.96%, where the P8 > P7 > P5 > P6 > P4 > P1 > P3 > P2. Generally, the amount of resistant starch produced after treatments depends on the botanical sources of the starch, heat treatment applied to the starch, amylose/amylopectin ration in starch, chain length of molecules, presence of amylose-lipid complex and others (Dupuis et al., 2014; Demirkesen-Bicak et al., 2018). A study by Nigudkar (2014) demonstrated that the RS content in two rice varieties was influenced by botanical origin, amylose and amylopectin ratio, cellular structure, and variation in the shape of starch granules and crystalline structure of the starch

In this study, the production of RS3 involved four sequential processing steps which are disruption of starch granules, enzymatic debranching of starch polymer, starch retrogradation and drying. The RS content for breadfruit RS3 samples of P1 and P6, P2 and P8, and P3 and P7 were compared based on methods of starch granules disruption (Table 1). From the results, it was portrayed that the used of distilled water for suspending the breadfruit starch significantly enhanced the production of RS3 compared to suspending the breadfruit starch in 0.1 M acetate buffer, pH 5. These results were in line with a study by Zi-Ni et al. (2015) on sago RS3 where the sago starch was suspended distilled water obtained the highest RS content (35.71%).

Apart from the factor above, the ways of starch's disruption also played an important role in RS3 formation (Dupuis et al., 2014). For this present study, boiling and autoclaving methods have been tested to all samples and the results for P1, P2 and P6, P8 were compared. The results revealed that method of starch gelatinization by boiling at 90°C for 30 minutes had significantly ($p <0.05$) enhanced the RS content in breadfruit RS3 sample compared to autoclave of breadfruit starch for 1 hour.

**Table 1.** Resistant starch (RS) content in breadfruit RS3 samples obtained from different processing conditions

| Sample | RS content (%) |
|---|---|
| P1 | $34.98 \pm 0.77^{c}$ |
| P2 | $28.96 \pm 0.84^{a}$ |
| P3 | $31.53 \pm 1.17^{b}$ |
| P4 | $36.65 \pm 1.51^{cd}$ |
| P5 | $43.11 \pm 1.53^{e}$ |
| P6 | $37.43 \pm 0.88^{d}$ |
| P7 | $50.13 \pm 2.03^{f}$ |
| P8 | $54.59 \pm 0.19^{g}$ |

Results are expressed as mean ± standard deviation (n=3).
Mean values in the same column followed by different superscript lowercase letters are significantly different ($p <0.05$).

Boiling is a type of cooking method which could increase the RS content of foods depending on the type of food. As was previously suggested by Eroglu and Buyuktuncer (2017), boiling method can increase the RS content in chickpeas and the increment of RS was due to the retrogradation of the starch that arises after boiling and gelatinisation process. In addition, Zi-Ni et al. (2015) have described in details the process of gelatinisation. It involved heating of the starch suspension in excessive of water. Then, the starch's temperature was elevated progressively, allowing starch molecules to absorb heat energy and increased the vibration which causes the breakage of hydrogen bonds among the starch molecules. Thus, the starch lost its crystallinity and then amylose chains leached out from the starch granules (Zi-Ni, 2015). At that time, it could be postulated that the starch gelatinisation by boiling is vital where a high amount of amylopectin could leach out from crystalline region of

the starch granules, and this has elevated the chances for pullulanase to react on branched amylopectin chains producing higher RS in samples.

In order to produce RS type III, enzymatic debranching treatment of the starch polymer using debranching enzyme, pullulanase was employed according to Ma et al. (2018). In this study, breadfruit starch samples were treated with pullulanase for 24 or 48 hours after the starch was gelatinised (Step 1). Using enzymatic treatments, RS3 was produced and the amount of RS was increased in breadfruit starch samples by increasing the levels of amylose via debranching of the amylopectin molecules (Dupuis et al., 2014). From Table 1, it shows that longer incubation time (48 hours) with pullulanase enzyme treatment had significantly reduced the RS content in breadfruit RS3 samples (comparing between P4 and P8 samples based on different enzyme incubation times). Hence, the results revealed that the longer the enzyme incubation time (48 hours), the higher the debranched amylose chains were produced, leading to the abundance of these chains in samples.

These excessive of amylose chains in samples would prevent the formation of RS as previously reported by Sajilata et al. (2006). In this study, 24 hours enzyme incubation time was an optimum period for the RS formation in breadfruit starch samples and it was in agreement with study by Zi-Ni et al. (2015) on sago RS3. Leong et al. (2007) also suggested that 16 and 18 hours of enzyme incubation time with pullulanase was the optimum time to increase the RS in sago sample instead of 0 to 8 hours, and did not significantly increase RS content in sago starch samples. Based on the results, it can be concluded that debranching of starch polymer with pullulanase had occured during the first 24 hours of the reaction and then remained constant even after prolonged the time of incubation and this reflected the time effective for the production of RS3.

Pullulanase is a debranching enzyme, hydrolysed α-1, 6-glucosidic bond of amylopectin in starch polymer which release short and long linear chains of amylose molecules (Chen et al., 2017). Therefore, along with the starch gelatinisation steps, availability of amylose molecules increased plus with recrystallization of amylose chains have enhanced the formation of RS3 in samples (Ismaiel et al., 2016). In this study, it was observed that dual autoclaving and retrogradation cycle might improves the RS content in breadfruit RS3 (comparing samples P1 and P5). This study was supported by Ai (2013), where dual autoclaving and retrogradation cycle have increased the formation of RS3 in corn starch from 21.3% to 40%. The short linear chains of amylose were participated in the rearrangement and recrystallization of starch (retrogradation) during autoclaving and the cooling treatment for RS3 (Ai, 2013).

*HCl- breadfruit RS3*

The highest RS content of breadfruit RS3 sample (P8 sample) (54.59%) was further purified with 0.5M HCl at 60°C for 24 hours, which then denoted as HCl-breadfruit RS3 according to Zi-Ni et al. (2015) method. Table 2 shows the RS content of breadfruit RS3 and HCl- breadfruit. Treatment of breadfruit RS3 with HCl was able to increase significantly ($p < 0.05$) the RS content from 54.59% to 57.86% (Table 2). This increment was due the acid hydrolysis method which used to purify the yield of RS3, where the hydroxonium ion ($H_30^+$) of acid hydrolysed the digestible portion and leaving the indigestible part of the breadfruit RS3 sample. Thus it was postulated that this method had contributed to increase the purity of the RS content of the HCl-breadfruit RS3. This result was in line with study by Zi-Ni et al. (2015) on sago RS3 using the same acid hydrolysis method which produced higher RS content in HCl-sago RS3. The digestible portion is the amorphous region while the indigestible portion refers to the crystalline region of the starch. Digestive enzymes have difficulty to access the crystalline regions, which created more resistance to digestion. This was explained by Zhang et al. (2014), stated that the higher crystalline structure formed in starch, the higher RS would be produced. Thus, this study revealed that acid hydrolysis could enhance the yield and purify the RS content of HCl-breadfruit RS3.

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**Table 2.** Resistant starch (RS) content of breadfruit RS3 and HCl- breadfruit RS3

| Sample | RS Content (%) |
|---|---|
| Breadfruit RS3 | 54.59 ± 0.19[b] |
| HCl- breadfruit RS3 | 57.86 ± 0.20[a] |

Results are expressed as mean ± standard deviation (SD), n = 3
Mean values in the same column followed by different superscript lowercase letters are significantly different ($p < 0.05$).

## Granules Morphology

Fig. 2 and 3 display the scanning electron micrographs of breadfruit starch, breadfruit RS3 and HCl-breadfruit RS3 viewed under SEM at magnifications of 300× and 500× respectively. The granular structure, sizes and surface characteristics of breadfruit starch and resistant starches (breadfruit RS3 and HCl-breadfruit RS3) were determined and it was observed that the granular structure of breadfruit RS3 and HCl-breadfruit RS3 had disappeared as compared to that of breadfruit starch for both magnifications. As for breadfruit RS3, it can be explained that after enzymatic treatments followed by retrogradation process, the smooth and granular structures were collapsed and the irregularly shaped particles formed for all samples (Demirkesen-Bicak et al., 2018).

Moreover, breadfruit starch shows the smooth and retained granular structures. Thus by having these structures, it can be characterised as resistant starch type II (RS2) as it has intact and remained granular structures of the starch. Therefore, the granule structures of breadfruit RS3 and HCl-breadfruit RS3 had indicated that both resistant starches were not in RS2 form (Zi-Ni et al., 2015). Besides that, the changes in appearance from granular to amorphous structure was likely due to the consequences of the starch gelatinisation where the coupled starch granules forms sponge-like structure, leading to double helix in the inner region of the retrograded starch (Reddy et al., 2015). Non-granular and retrograded starches are some of the characteristics for RS3 (Zhang et al., 2014). These observations are consistent with those reported for lotus seed resistant starch (Zhang et al., 2014).

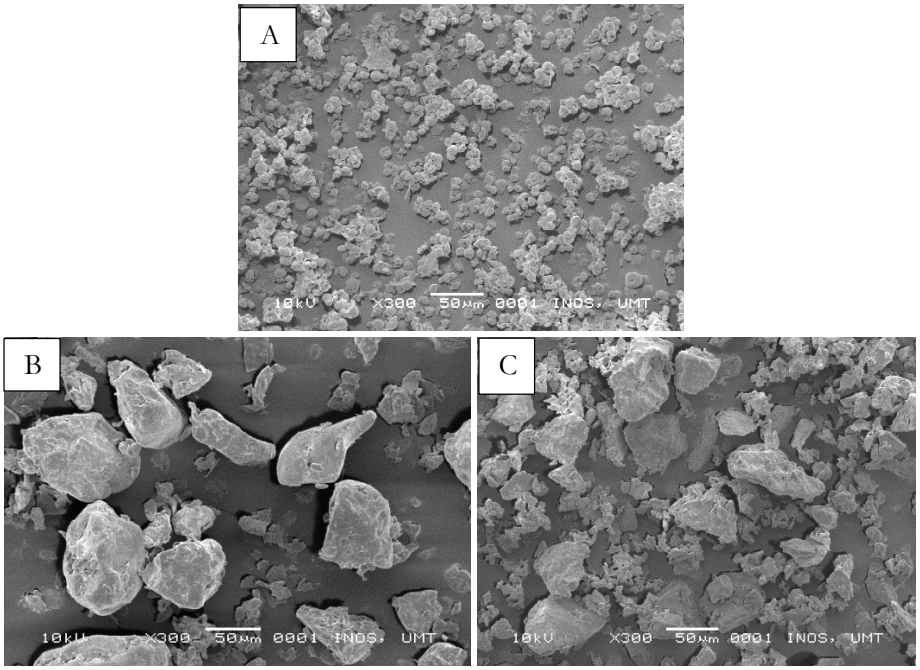


**Fig. 2.** Scanning electron micrographs of (A) breadfruit starch, (B) breadfruit RS3 and (C) HCl-breadfruit RS3 at 300× magnifications viewed under SEM.

Furthermore, breadfruit RS3 and HCl-breadfruit RS3 have irregular shape with a continuous sponge-like and porous structure. Similarly, those resistant starches also resembled an amorphous structure which caused by the loss of granular appearance, leached out the amylose chains from the starch granules, loss of the amylopectin crystalline region during autoclaving, and re-association of the starch chains within the granules during the production of RS3 (Zhang et al., 2014). These results were in line with a study by Reddy et al. (2015) on the structure of retrograded potato starch.

As indicated in Fig. 3, it was observed that granules of breadfruit RS3 have smoother outer surface and bigger in size (>50 μm) as compared to HCl-breadfruit RS3 which had rougher outer surface and smaller in size of the starch granules. This result was in contrast with Zi-Ni et al. (2015) for sago RS3, where the surface obtained from acid hydrolysed sago RS3 was smoother as compared to non-hydrolysed sago RS3. This might due to characteristic variations from different starch sources. Meanwhile, the granules of HCl-breadfruit RS3 were appeared to have smaller fractions compared to breadfruit RS3 and it may come from reaction of acid hydrolysis on breadfruit RS3. Acid hydrolysis degraded the amorphous region of breadfruit RS3, releasing smaller starch fractions and remained the starch crystalline structure in HCl-breadfruit RS3 (Zhang et al., 2014)

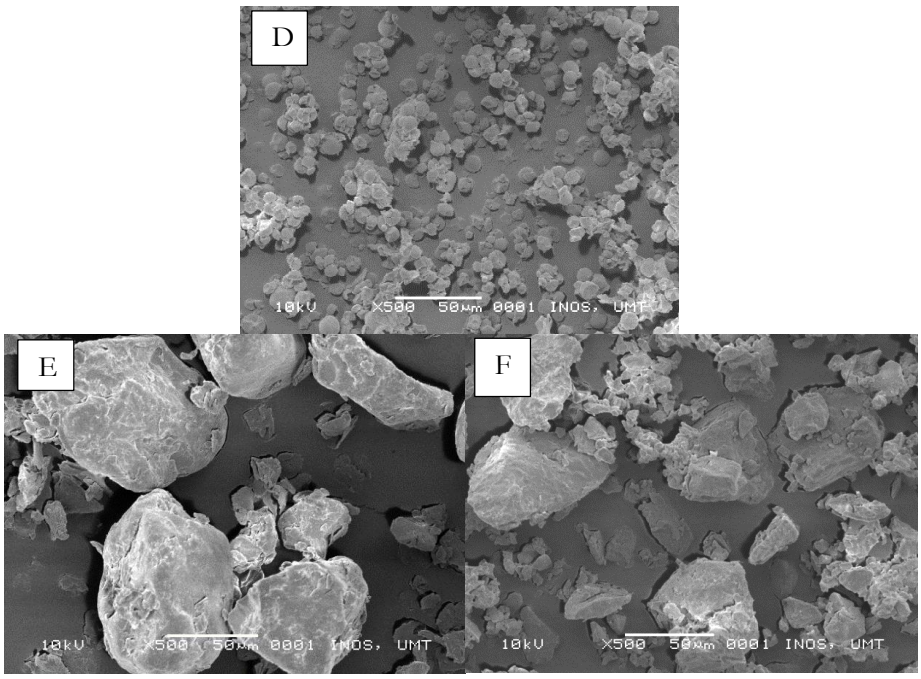


**Fig. 3.** Scanning electron micrographs of (D) breadfruit starch, (E) breadfruit RS3 and (F) HCl-breadfruit RS3 at 500× magnifications viewed under SEM.

## CONCLUSION

In summary, different processing conditions of the breadfruit starch have influenced the RS content and granule morphology of the breadfruit RS3 produced. Breadfruit RS3 sample, (P8) obtained from the processing 8 produced the highest RS content (54.59%) as compared to other RS3 samples. This pre-studied had selected the best processing conditions, processing 8 for RS3 production that involved the gelatinisation of the starch suspension using distilled water by boiling method at 90°C for 30 minutes, followed by pullulanase debranching at 60°C for 24 hours, single thermal (autoclave) and cold storage cycle at 4°C for 24 hours. P8 sample was further purified using acid hydrolysis with 0.5 M HCl acid at 60°C for 24 hours which was then produced HCl-breadfruit RS3 with 57.86% of RS content. Treatment with acid hydrolysis has increased the RS content and

produced finer and smoother surface with more crystalline region in HCl-breadfruit RS3 as compared to other samples.

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## REFERENCES

Ai, Y. (2013). Structures, properties, and digestibility of resistant starch. Ph.D Thesis (pp 1-177). Iowa State University.

Appiah, F. & Oduro, I. (2011). Functional properties of *Artocarpus altilis* pulp flour as affected by fermentation. *Agriculture and Biology Journal of North America, 2*(5)**,** 773–779.

Chen, M. H., Bergman, C. J., McClung, A. M., Everette, J. D. & Tabien, R. E. (2017). Resistant starch: Variation among high amylose rice varieties and its relationship with apparent amylose content, pasting properties and cooking methods. *Food Chemistry, 234*, 180–189.

Demirkesen-Bicak, H., Tacer-Caba, Z. & Nilufer-Erdil, D. (2018). Pullulanase treatments to increase resistant starch content of black chickpea (*Cicer arietinum L.*) starch and the effects on starch properties. *International Journal of Biological Macromolecule, 111***,** 505-513.

Dhita, S., Warren, F. J., Butterworth, P. J., Ellis, P. R. & Gidley, M. J. (2009). Mechanism of starch digestion by α-amylase–structural basis for kinetic properties. *Bioorganic & Medicinal Chemistry Letters, 19*(10)**,** 171.

Dupuis, J. H., Liu, Q. & 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.

Eroglu, E. I. & Buyuktuncer, Z. (2017). The effect of various cooking methods on resistant starch content of foods. *Nutrition and Food Science, 47*(4), 522–533.

Fuentes-Zaragoza, E., Riquelme-Navarrete, M. J., Sánchez-Zapata, E. & Pérez-Álvarez, J. A. (2010). Resistant starch as functional ingredient: A review. *Food Research International, 43*(4), 931–942.

Fuentes-Zaragoza, E., Sánchez-Zapata, E., Sendra, E., Sayas, E., Navarro, C., Fernández-López, J. & Pérez Alvarez, J. A. (2011). Resistant starch as prebiotic: A review. *Starch -Stärke, 63*(7), 406–415.

Gao, Q., Li, S., Jian, H. & Liang, S. (2011). Preparation and properties of resistant starch from corn starch with enzymes. *African Journal of Biotechnology, 10*(7), 1186–1193.

Goni, I., Garcia-Diz, L., Manas, E. & Saura-Calixto, F. (1996). Analysis of resistant starch a method for foods. *Food Chemistry, 56*(40), 445–449.

Ismaiel, M., Yang, H. & Min, C. (2016). Dietary fiber role in type 2 diabetes prevention. *British Food Journal, 118*(4), 961–975.

Leong, Y. H., Karim, A. A. & Norziah, M. H. (2007). Effect of pullulanasedebranching of sago (*Metroxylon sagu*) starch at subgelatinization temperature on the yield of resistant starch. *Starch/Staerke, 59*(1), 21-32.

Ma, Z., Hu, X. & Boye, J. I. (2018). Research advances on the formation mechanism of resistant starch type III: A review. *Critical Reviews in Food Science and Nutrition*, 1–22.

Madzlan, K., Hasnisa, H., Sabeetha, S. & Dayana, M. N. (2012). Extraction of starch and enzymatic production of high amylose starch from sweet potato (*Ipomea batatas*) var. Telong. *Journal of Tropical Agriculture and Food Science, 40*(2), 203–210.

Nigudkar, M. R. (2014). Estimation of resistant starch content of selected routinely consumed Indian food preparations. *Current Research in Nutrition and Food Science, 2*(2), 73–83.

Nochera, C. & Ragone, D. (2016). Preparation of a Breadfruit Flour Bar. *Foods, 5*(4), 37–43.

Nor, M. Z. M., Talib, R. A., Noranizan, M. A., Chin, N. L. & Hashim, K. (2014). Increasing Resistant Starch Content in Fish Crackers through Repetitive Cooking-Chilling Cycles. *International Journal of Food Properties, 17(*5), 966–977.

Nwokocha, L. M. & Williams, P. A. (2011). Comparative study of physicochemical properties of breadfruit (*Artocarpus altilis*) and white yam starches. *Carbohydrate Polymers, 85(*2), 294-302.

Öztürk, S. & Mutlu, S. (2018). Physicochemical Properties, Modifications, and Applications of Resistant Starches. In *Starches for Food Application* (pp. 297–332). Elsevier Inc.

Reddy, C. K., Pramila, S. & Haripriya, S. (2015). Pasting, textural and thermal properties of resistant starch prepared from potato (*Solanum tuberosum*) starch using pullulanase enzyme. *Journal of Food Science and Technology, 52*(3), 1594–1601.

Sajilata, M. G., Singhal, R. S. & Kulkarni, P. R. (2006). Starch — A Review. *Comprehensive Reviews in Food Science and Food Safety, 5*, 1–17.

Zhang, Y., Zeng, H., Wang, Y., Zeng, S. & Zheng, B. (2014). Structural characteristics and crystalline properties of lotus seed resistant starch and its prebiotic effects. *Food Chemistry, 155*, 311- 318.

Zi-Ni, T., Rosma, A., Karim, A. A. & Liong, M. T. (2015). Functional properties of resistant starch type-III from *Metroxylon sagu* as affected by processing conditions. *Pertanika Journal of Tropical Agricultural Science, 38*(3), 399–412.

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