Resistant starch content, pasting properties, and structure of modified taro (Colocasia esculenta L. Schott) starch granule by steam cooking

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Abstract. Effect of steam cooking (SC) on resistant starch (RS) content, pasting properties, and structure of modified taro starch granule was studied. Modified starch was prepared by two cycles of autoclaving followed by cooling at 4, and -20°C. The RS content was significantly higher (p < 0.05) after SC treatment as compared with native taro starch. All modified starches tend to be more stable to SC and heat- and shear-resistant of pasting characteristic which was confirmed by RVA viscograme. Moreover, the structure of modified taro starch granules showed remarkable different as compared to native as shown by the irregular and fracture of the structure.

1. Introduction

Various number of the modern food industry need superior ingredients, including starches, which is tolerate in the various processing conditions [1]. One of the prospective alternatives is by modifying starch to form indigestible residues which is more durable and resistant to various condition including heat, acid, and shear stress, known as resistant starch (RS) which has similar physiological function to dietary fiber.

RS is a starch fraction that is indigestible in the human digestive tract and then fermented in the colon by microorganisms. It is also considered as dietary fiber due to its characteristic [2]. Previous studies reported that increased RS consumption had beneficial effects on glycemic and insulin response so that increasing dibetes risk [3,4]. The beneficial properties of RS also could be considered as an superior ingredient of functional food products, particularly for steamed and heated foods due to its thermostability. Several food products made with RS have been developed including instant noodles [5], bihon-type noodles [6], and rice noodles [7]. One of starch-rich tubers that could be modified as RS candidate is Taro (Colocasia esculenta L. Schott) which is still under utilized in Indonesia.

Steaming is a food processing method which is widely used and popular in the food industry and home preparation and will destruct the starch granules which in turn altering its characteritics such as RS content, structure of starch granule, and pasting properties. However, there is still limited information on this study. Hence, the effect of steam cooking on resistant starch content, pasting properties, and granule morphology of taro modified starch was conducted.
2. Materials and Methods

Materials
Commercial taro starch, Hasil Bumiku brand, purchased from local wholesale market in Bantul, Yogyakarta, Indonesia was used in this study. RS assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland) was used to analyze the RS content.

Preparation of modified starch
Modified taro starch was produced according to the modification of previous study [8]. Taro starch was blended with distilled water based on the ratio 1:3.5, and the blend was then gelatinized using pressure-heated instrument at temperature 121°C for 30 minutes and cooled in various temperatures (4, and -20°C) with repetition of twice cycles. Afterwards, the retrogradated starch was dried using fan assisted oven at temperature 60°C for 16 hours after allowed to cool at room temperature (29 ± 2°C) for 24 hours, and it was subsequently grounded, sieved using a 60 mesh.

Preparation of SC
Both native and modified taro starch were added and blended with distilled water with ratio 4:3 and then flattened using press roller. After steam cooking for 10 minutes, the doughs were dried in fan assisted oven at temperature of 60°C for 2 hours. Afterwards, the samples were grinded and sieved using a 60 mesh.

RS content
RS content was measured based on Megazyme Resistant Starch Assay Kit (Megazyme International, Wicklow, Ireland), AACC method 32-40 [9]. Both sample (100 ± 5 mg) and 4 mL of enzyme mixture (pancreatic α-amylase and amyloglucosidase) were poured to each test tube, vortexed and kept in a shaker water bath for 16 hours at temperature of 37°C (200 strokes/min) to hydrolyze digestible starch. Afterwards, the suspension was added 4 mL absolute ethanol and mixed to deactivate the enzymes. Resulting pellet from suspension centrifugation (5000×g, 10 minutes) was subsequently washed by using 50% ethanol twice to remove the digested starch. The sediment was dissolved in 2 mL of 2 M KOH by vigorously stirring for 20 minutes and neutralized by adding 8 mL sodium acetate buffer (1.2 M). Then it was added and blended with amyloglucosidase (0.1 mL, 3300 U/mL) and stored in a water bath at temperature 50°C for 30 minutes, and centrifuged at 3000 × g for 10 minutes. Glucose-oxidaseperoxidase-aminoantipyrine (GOPOD) 3 mL was added and mixed to 0.1 mL of the supernatant, and it was kept at 50°C for 20 minutes. Spectrophotometer at 510 nm was selected to measure the sample absorbance. RS content was calculated by using following formula:

$$RS (g/100 \, \text{g sample}) = \Delta E \times F/W \times 90$$

Where, ΔE is absorbance (reaction) read against the reagent blank, F is conversion from absorbance to micrograms (the absorbance obtained for 100 µg of D-glucose in the GOPOD reaction is determined, and F = 100 (µg of D-glucose) divided by the GOPOD absorbance for this 100 µg of D-glucose, and W is dry weight of sample analyzed.

Pasting properties
Pasting properties of the samples were determined using instrument Rapid Visco Analyser (RVA 4500, Perten Instruments, PerkinElmer Inc) following AACC Standard method No.61-02 (AACC, 2000).

Morphology of starch granule
Morphology of starch granule samples were observed using Scanning Electron Microscopy (SEM). All gold coated samples were then scanned by using a SEM (Shimadzu SSX-550). An voltage of 20 kV and magnification of 1500x were used.
Statistical analyses
The results are expressed as mean ± standard deviation of duplicate determinations. Analysis of variance
was used to determine the least significant at p < 0.05 using Statistical Package for Social Scientists
(SPSS, version 16.0). DMRT test was then performed to evaluate differences by means of the samples.

3. Results and Discussion
Effect of SC on the yield of RS
An alteration was observed after SC in the RS content of the samples, showed in Table 1. The RS
yields of the SC samples were significantly (p < 0.05) higher than those of the initial samples, except
SC native. A similar finding was earlier stated by Ghugre et al. (2013) wherein RS content enhancement
of sago starch could be associated to the forming of crystalline regions by amylose recrystallization in
room temperature after cooking [10]. Cooling in various temperatures (4, and -20°C) applied in modified
starch could not alter the RS yield significantly (p < 0.05). However, those were different with SC samples wherein RS yields were significantly higher than SC native. The increase in the RS content of modified starch might be due to repeated and prolonged heating-cooling. Heating allow the starch to gelatinize and release of more amylose molecules. Furthermore, cooling lead to reassociation of polymer chains resulting better retrogradation of starch as indicated by Cheung and Chau (1998) and Song et al. (2012) [11,12].

Table 1. Resistant starch yields of native and modified starch before and after SC

| Sample     | RS yield (%) |
|------------|--------------|
| Native     | 0.75 ± 0.02bc|
| 4°C        | 0.88 ± 0.05bc|
| -20°C      | 1.01 ± 0.02b |
| SC native  | 0.57 ± 0.04c |
| SC 4°C     | 5.12 ± 0.10a |
| SC -20°C   | 5.18 ± 0.12a |

Means followed by different letters indicate statistically significant differences (p < 0.05).

Pasting properties
Figure 2 and Table 2 represents pasting characteristics of native and modified starch before and after
SC. It confirm the functional behavior of samples during heating and cooling periods, which is widely
used for processing of starchy products or starch-based ingredient [13]. The peak viscosity, trough,
breakdown of modified starch are lower than the native starch for SC samples compared to non SC.
Those decrement confirm that that SC treatment is able to alter the starch granule to be stronger and
more resistant to swell at high temperature. In addition, the lower breakdown value of SC samples
indicate that it is more durable to heat and shear stress. While, the setback properties of SC tend to
increase which indicate starch retrogradation tendency after gelatinization. When comparing the
influence of SC treatment on pasting properties of the samples, modified starch cooled at -20°C tend to
more stable rather than native and another modified one. Furthermore, based on the RVA viscoagrame,
those of modified starch became heat- and shear-resistant during pasting which indicate the
compatibility as a thermostable ingredient.
Figure 1. The pasting curves of native and modified starch before and after SC.

| Sample | Peak (cP) | Trough (cP) | Breakdown (cP) | Final Viscosity (cP) | Setback (cP) | Peak Time (min) |
|--------|-----------|-------------|----------------|----------------------|--------------|-----------------|
| Native | 2958.00   | 2505.00     | 453.00         | 3021.00              | 363.00       | 7.00            |
| 4°C    | 1480.00   | 1443.00     | 37.00          | 376.00               | 124.00       | 7.00            |
| -20°C  | 234.00    | 218.00      | 16.00          | 1839.00              | 96.00        | 6.87            |
| SC native | 896.00 | 681.00      | 215.00         | 1044.00              | 516.00       | 7.00            |
| SC 4°C | 207.00    | 175.00      | 32.00          | 299.00               | 396.00       | 6.13            |
| SC -20°C | 224.00  | 205.00     | 19.00          | 301.00               | 158.00       | 6.93            |

Structure of starch granule

SEM was set at the magnification of 1500x to observe the native and modified starch granules before and after SC which are shaped tend to polygonal shape (Figure 3). It is in agreement with previous reports on taro starch granules had polygonal and irregular shapes [14]. The native starch granule both before and after SC had a smaller size compared to the modified ones. After modification, the granular shapes remarkably changed. Also, SC treatment was able to alter starch granule morphology. The structure of modified and SC starch were completely destroyed to form a more compact, irregular and fracture structure. This could be attributed to the result of heat and moisture used in modification and SC method, which caused some swollen or even completely gelatinized granules followed by retrogradation, which is reorganization of the starch chain into a helical complex and strengthened by hydrogen bonds [15].
4. Conclusion

This study concludes that the effective modification for increasing of RS content was by two cycles of autoclave-cooling (4°C and -20°C). Moreover, lower cooling temperature (20°C) resulted in a more heat- and shear- stable. Furthermore, SC processing exhibited an alteration to the starch granule morphologies. Those characteristics show a promising alternative thermostable ingredient which is beneficial for development of functional food products.

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