

# Effect of Autoclaving-Cooling Treatments on Chemical Characteristic and Structure of Tacca (*Tacca Leontopetaloides*) Starch

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## ABSTRACT

Tacca tubers (*Tacca leontopetaloides*) was used to produce many kind of foods but has limit in utilization. Tacca tubers has a high starch and amylose content which can be a functional food by increasing the resistant starch content. The objective of this research was to evaluated the effect of autoclaving-cooling treatments on chemical characteristic and structure of tacca starch. Tacca starch was modified by autoclaving-cooling in 1-5 cycle(s). Then, analysis of amylose and resistant starch content of the starch. An observation was done to the structure of the starch granules of samples using Scanning Electron Microscope. The results showed that the autoclaving-cooling significantly increased the resistant starch content from native starch 4,25 % to 39-53%, while the amylose content did not significantly increased. The microstructure of the starch granules have changed from coccus shape into crystalline structure through SEM observation.

**Keywords:** Tacca starch, resistant starch, amylose content, autoclaving-cooling, SEM

## I. INTRODUCTION

Indonesia has many natural resources which potential to develop, such as tubers, as a source of carbohydrates. Tacca tuber (*Tacca leontopetaloides*) has grow in the south coast, especially in Garut Regency, West Java. Tacca Starch has a high starch content that is equal to 66.65% with amylopectin content of 43.88% and amylose 22.77% [1]. Amylose and amylopectin content can be used to increase the resistant starch content so that it can increase the functional value of starch. Increasing the content of resistant starch can be done by modifying the starch through physical, chemical or enzymatic treatment. Modification will cause changes in the structure and nature of starch [2]. One treatment for physical modifying starch is by autoclaving-cooling. Several studies were obtained on the starch modification by the autoclaving-cooling methods that increase the levels of resistant starch [3,4,5,6]. The autoclaving-cooling method can cause starch structure changes into crystalline particles [7]. However, tacca starch modification with autoclaving-cooling is not reported yet. This research aim was to determine the effect of autoclaving-cooling treatment on chemical characteristic and structure of tacca starch.

## II. METHODS

### A. Materials

Tacca tuber was obtained from the south coast of Garut District, West Java. The materials used for this research

included autoclave, refrigerator, cabinet dryer, analytical balance, orbital incubator shaker (Stuart SI500), Centrifuger-BL-II, vortex, elisa reader (Thermo Scientific), pH meter, oven, Scanning Electron Microscope (SEM) (Hitachi SU 3500), and glassware. The chemicals used for the analysis included pepsin enzyme,  $\alpha$ -amylase enzyme, amiloglucosidase enzyme, KCl-HCl buffer pH 1.5, Trismaleate buffer 0.1 M pH 6.9, sodium acetate buffer 0.4 M pH 4.75, 4 M KOH solution, 2 M HCl, aquadest, glucose assay kit (sigma GAGO-20), 95% ethanol solution, 1 N NaOH, 1 N acetic acid, and 0.01 N iodine.

### B. Production of Tacca Starch

Tacca tubers were peeled, washed with clean water, slices into small pieces and grinded using a grinder. Then, add water, filtered, and incubation at room temperature during 24 hours to precipitate the starch. The starch obtained then washed using water and dried using a cabinet dryer.

### C. Autoclaving-cooling cycle treatment

Tacca starch was dissolved with distilled water to 30% w/v distilled water, heated until homogeneous and thickens, then autoclaved for 15 minutes at 121° C. The next process was cooling in a refrigerator at 4°C for 24 hours. The autoclaving-cooling process was done as much as 1, 2, 3, 4, and 5 cycle. Then, dried using a cabinet dryer at 50-60°C for 24 hrs, milled and sieved (100 mesh size).

#### D. Analysis

Chemical analysis were determined to the tacca starch, including resistant starch content [8], amylose content with iodo calorimetry method [9], and water content [10]. Structure analysis of tacca starch was observed using Scanning Electrone Microscopy (SEM).

#### E. Statistical Analysis

Statistical analysis was performed using SPSS 16 software. Data were analyzed by one-way ANOVA and continued with Duncan Multiple Range Test with a significance level of 5%.

### III. RESULTS AND DISCUSSION

#### A. Chemical Analysis

Resistant starch of tacca starch was increased significantly with modify the process by autoclaving cooling method. The results of this research are in agreement with the other studies about increasing resistant starch level using autoclaving-cooling method [6]. During the autoclaving

process, starch gelatinization was occurred which results in the release of starch molecules from the starch granules, and gradually break down. Whereas during the cooling process, starch retrogradation was occurred, re-association of the polymer chain (the process when amylose with amylose and amylose with amylopectin binds to each other) into a double helical stabilized with hydrogen bonds, resulting in starch structure becoming more compact and difficult to hydrolysis by digestive enzymes [11].

The autoclaving-cooling modification of several cycles in tacca starch did not show any significant difference in resistant starch levels. This obtained may occurred because the gelatinization process requires excess water which will cause swelling of the starch granules so that amylose can come out. Therefore, treatment with several heating cycles requires more water. The less amount of water causes less disruption of the amylose helical structure in the next cycle so that the amount of amylose that comes out of the starch granule was not optimum. Then, the number of amylose-amylose and amylose-amylopectin bonds which binding again was less so that the RS formed was lower in the next cycle [3].

**Table 1. Resistant starch and amylose content of tacca starch**

Sample	Resistant starch (%)	Amylose content (%)
Natural tacca starch	4.25 <sup>a</sup> ± 0,2	30.5% <sup>ab</sup> ± 6,9
Tacca starch AC 1 cycle	41 <sup>b</sup> ± 9,6	30% <sup>ab</sup> ± 5,35
Tacca starch AC 2 cycle	53 <sup>b</sup> ± 14,13	39,25% <sup>b</sup> ± 21,49
Tacca starch AC 3 cycle	42 <sup>b</sup> ± 23,67	20,7% <sup>a</sup> ± 4,65
Tacca starch AC 4 cycle	39 <sup>b</sup> ± 7,2	23,5% <sup>ab</sup> ± 8,61
Tacca starch AC 5 cycle	40 <sup>b</sup> ± 6,37	26% <sup>ab</sup> ± 2,12

Note : The same superscript symbol in the same column indicate that sample are not significantly different at a significance level of 95%

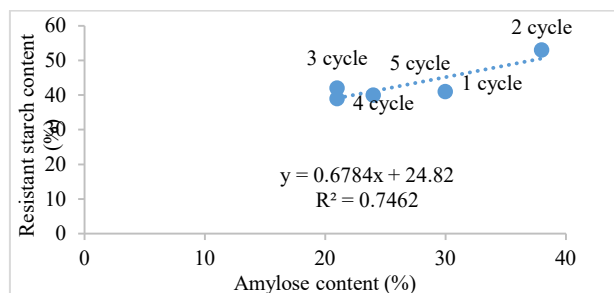
The autoclaving-cooling modification of several cycles in tacca starch did not show any significant difference in resistant starch levels also due to the fact that the starch has been completely gelatinized and metrogradated in the first cycle of autoclaving-cooling treatment so that in the next cycle did not experience significant changes in resistant starch content.

The autoclaving-cooling process did not have a significant effect on amylose content of tacca starch. The autoclaving-cooling treatment does not provide a significant different because autoclaving is a physical treatment that does not have a significant effect on chemical changes in starch [12]. Similar results were also obtained by the other researcher [12,13] who modified rice starch and potato starch. The process of modifying starch using autoclaving-

cooling with several cycles requires a greater amount of water. If the amount of water is lacking, it is likely to disrupt the helical structure of the amylose in gelatinization of the next cycle so the amylose content that comes out of the granule is not optimum. Autoclaving-cooling process of one cycle causes the amylose contained in natural starch to experience retrogradation of starch to form amylose complexes that are resistant to digestive enzymes. Besides that, it is possible to have a reaction between amylose and fat so that it becomes an amilo-lipid complex which is a type 5 resistant starch or there is a reaction between amylose and protein so that it becomes a amilo-protein complex [3].

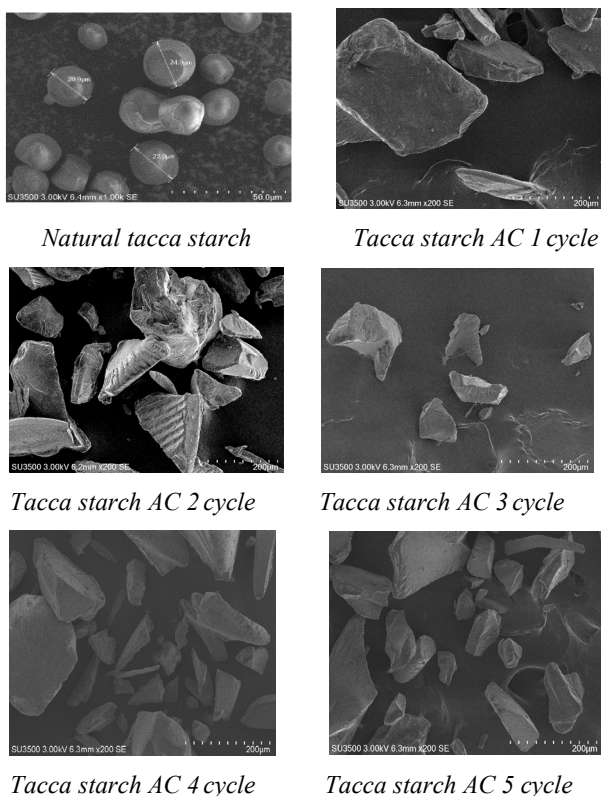
Increased levels of resistant starch can be influenced and closely related to the amylose content in the sample, as

shown in Figure 1. The autoclaving-cooling treatment with 1 to 5 cycles gives an effect on the levels of resistant starch and amylose although it does not show a significant different. The correlation graph between amylose and resistant starch have a positive correlation value of 0.7462, indicated a high or strong correlation. The autoclaving process can cause a hydrolysis process that will break the glycosidic linkage of amylopectin to amylose and the termination of the polysaccharide chain so that the short chain of polysaccharides will increase.



**Figure 1. Correlation Graph between Resistant Starch Levels and Amylose of Content of modified starch using autoclaving-cooling method**

### B. Structure of Tacca Starch



**Figure 2. Scanning electron microscopy of tacca starch with 3000x magnification**

The structure of natural tacca starch and modification can be seen in the figure 2. Natural tacca starch has round

starch granules with a diameter of 20.9-24.0  $\mu\text{m}$  and smooth surface. Tacca starch has an oval starch granules and small size [14,15]. The autoclaving-cooling treatment causes the change of tacca starch structure. The structure of the starch granules becomes shapeless and large complexes forms with uneven surfaces. Autoclaving-cooling modification caused the shapeless granules structure and the presence of small holes on the surface of the starch crystals [7]. In the autoclaving process, the starch granule structure is damaged and swollen so that it is irregular in shape. Then in cooling process, starch retrogradation occurs, which gelatinized starch will be converted into crystals, where the crystalline form will be resistant to digestive enzymes [16].

## IV. CONCLUSION

Physical modification of tacca starch by autoclaving-cooling treatment can significantly increase resistant starch. The most optimal autoclaving-cooling cycle is autoclaving-cooling with 1 cycle. The autoclaving-cooling treatment does not have a significant effect on amylose content. Amylose content has a strong correlation with resistant starch levels due to autoclaving-cooling on starch. The autoclaving-cooling treatment causes the change of starch granules from round to formless and form large crystal complexes with uneven surfaces.

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