Effects of various cooling temperatures on resistant starch formation and pasting behaviour of autoclaved taro flour

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Abstract. Taro is one of the tubers rich in starch which is prospectively improved for its functional properties. In this work, the cooling treatment subjected to the autoclaved taro flour on resistant starch formation and pasting behaviour were evaluated. The flours were autoclaved at 121°C for 30 minutes followed by cooling at different temperatures (25, 4, and -20°C) and repeated twice cycles. Among the cooling treatments, the 4°C had the highest RS (13.11%) compared to 25°C and 4°C which were 13.02% and 12.76% respectively. The autoclaved-cooled treatment altered the microstructure of starch granules showing fracture morphology and irregular shape. The starch morphology treated by autoclaving-cooling at the lowest temperature exhibited a slightly rougher and more wrinkled than other cooling temperatures. Moreover, repeated autoclaving-treatment lowered the pasting temperature. Other than the cooled flour at 25°C, the pasting parameters of the treated flours (peak, final viscosity, and setback) were higher than the untreated flour.

1. Introduction
Consumption trend of carbohydrate sources in human diet is today not only for source of energy, but also to obtain its health-related benefits. For the beneficial effect purposes, starch is usually modified to form indigestible portion which is more resistant to absorb and digest along the gastrointestinal tract [1]. Resistant starch (RS) has similar physiological function to dietary fiber which may be fermented in the colon either completely or partially [1, 2]. Furthermore, enhancement of RS yield showed useful properties for food application because of the thermostability, viscosity, and shear resistance [3, 4].

Taro (Colocasia esculenta L. Schott), a starchy tuber, is potentially developed into RS. Taro is broadly found in tropical countries including Indonesia which many Indonesians process it into noodles, cakes, chips, and others. Several types of RS have been classified including physically inaccessible starch (RS type 1), resistant starch granules (RS type 2), retrograded starch (RS type 3), and chemically modified starch (RS type 4) [1]. The RS preparation methods have been widely studied including enzymatic and acid treatment, chemical modification, and hydrothermal-retrograded technique. In order to result RS 3, retrograded starch from cooking-cooling method, gelatinization followed by retrogradation step is needed [5, 6]. Physical method by using autoclaving-cooling treatment is commonly applied to produce RS 3 because of its convenience and relatively low cost.

It has been known that cooling temperature improves the RS yield enhancement beside gelatinization temperature. Cooling temperature is used for retrogradation which causes amyllose crystallization facilitated by hydrogen bond. During storage at lower temperature, the molecules undergo re-association resulting more tightly packed structures [7]. However, there is no information
on the effect of various cooling temperatures on RS formation of taro. Hence, further research concerning the effects of cooling temperature on RS yields and pasting behaviour of taro flour need to evaluate.

2. Materials and Methods

2.1. Preparation of RS
RS 3 was prepared following Zhao and Lin method (2009) with slight modification. Taro flour (Naya brand, Bogor): distilled water (1:3.5) was mixed and subsequently gelatinized using autoclave at temperature 121°C for 30 minutes [8]. The flour paste was cooled in various temperatures (25, 4, and -20°C) for 24 hours aimed to allow retrogradation process after the flour paste temperature was conditioned at 29 ± 2°C. That preparation was repeated by twice cycles. The retrograded flour was then dried by using fan equipped oven at 60°C for 16 hours. Afterwards, it was grounded and followed by sieving machine in a 60 mesh.

2.2. RS content, morphology, and pasting behaviour
Evaluation of RS content was conducted by in vitro testing referring to Goñi et al. (1996) [9]. For starch granule morphology, the samples coated by gold were observed using Scanning Electron Microscopy (SEM). SEM (Shimadzu SSX-550) was used at voltage of 20 kV and magnification of 5000x, and 10000x. Rapid Visco Analyser (RVA 4500, Perten Instruments, PerkinElmer Inc) was selected for evaluating the sample pasting properties following the AACC Standard method No.61-02 (AACC, 2000). The parameters measured consisted of pasting temperature, peak viscosity, trough viscosity, breakdown, final viscosity, and setback.

2.3. Statistical analysis
The results of those testing were statistically evaluated by analysis of variance (ANOVA) and followed by Duncan’s Multiple Range Test (DMRT) using p < 0.05 when needed.

3. Results and Discussion

3.1. Effect of cooling temperature on the yield of RS
The RS content of taro flours, which were modified by autoclaving-cooling at 25, 4, and -20°C are presented in Figure 1. The RS content of each sample were statistically (p < 0.05) different where 4°C sample has the highest yield (13.11%). Enhancement of RS content of starch was attributed to the crystalline formation from amylose recrystallization caused by storage at low temperatures after gelatinization treatment. Moreover, gelatinized starch stored at freezing temperatures lead to the lower retrogradation. It was confirmed by previous findings that frozen storage was able effectively inhibit starch retrogradation due to a forming of the more rigid glass matrix and less molecular mobility resulting retardation of amylose reassociation [11, 12].

![Figure 1. RS yield of the autoclaved-cooled taro flours.](image-url)

3.2. Starch granule morphology
SEM is applied to observe the starch granule morphology by using magnification of 5000, and 10000x showed in Figure 2. The granules of the native starch tend to be polygonal shape with smooth surface which is similar trend to the previous studies on starch granule of taro [13,14]. After treatment of starch gelatinization, the changes in the granule appearance of fracture morphology and irregular shape were displayed. This may be ascribed as the impact of autoclaving-cooling treatment resulting some swollen or gelatinized granules around the surface [15]. Alteration of starch granule morphology could be associated to the retrogradation of amylose chains which are stabilized by hydrogen bonds. Furthermore, the morphology of autoclaved-freezed starch appeared to be slightly rougher and more wrinkled than other treatments. The aggregation of starch molecule fragments was possibly more occurred in the very low temperature. Compared to autoclaved-chilled, the morphology of autoclaved-cooled starch granules tends to be similar with a noticeable rough oval shape.

![Figure 2](image.png)

**Figure 2.** Starch granule morphology of the native and autoclaved-cooled taro flours.

### 3.3. Pasting properties

The pasting properties of the native and autoclaved-cooled flours are summarized in Table 1 and Figure 3. Autoclaving treatment and cooling temperature influence the pasting behaviour of the native flour. Pasting temperature represents a minimum temperature needed for cooking in which indicate energy required for production process [16]. The lowering of autoclaved-cooled flours was probably associated with the partial cleavage of the glycosidic bonds due to high temperature exposure of autoclave. Other RVA parameters of modified flour of taro were higher than the native one except for the modified flour cooled at 25°C. The decrease in peak viscosity, that is achieved because of a temperature enhancement after pasting temperature, of 25°C sample may be attributed to the degradation of starch granules because of heat combined pressure during autoclaving [6]. Consequently, starch molecular reorganization is decreased resulting a lower of hydration and swelling capacity. Otherwise, the higher peak viscosity exhibited by -20 and 4°C may be associated with their higher swelling power compared to the native and 25°C sample. The same trend occurred
for final viscosity, which the temperature treated constantly while the viscosity increases until a plateau is achieved. Further, very important characteristics attributed to the RS yield, setback, was also affected by the autoclaved-cooled process. Setback represents the enhancement of viscosity due to the cooling stage of starch granules so that retrogradation occurred.

![Figure 3. Pasting behaviour of native and modified starch.](image)

| Sample | Pasting temperature (°C) | Peak (cP) | Final Viscosity (cP) | Setback (cP) |
|--------|--------------------------|-----------|----------------------|--------------|
| Native | 83.90                    | 911.00    | 1239.00              | 625.00       |
| -20°C  | 67.70                    | 1408.00   | 1657.00              | 726.00       |
| 4°C    | 77.35                    | 1467.00   | 2101.00              | 962.00       |
| 25°C   | 51.80                    | 663.00    | 1081.00              | 564.00       |

**4. Conclusion**

The cooling temperatures on autoclaved taro flour affected the RS content and pasting behaviour. The cooling treatment at 4°C exhibited the highest RS yield comparing with other cooling temperatures (-20°C and 25°C). There was no sign of fractures were showed on the surface of the untreated starch granules confirmed by SEM. While the treated starch granule resulted in some cracks and irregular shape which may correspond to the repeated gelatinization. Furthermore, compared to native taro flour, pasting behaviour of autoclaved-cooled flours shifted to the lower temperature. Other pasting parameters of modified flours demonstrated higher value than the untreated flour except for the autoclaved flour and cooled at 25°C. Additional investigation should be performed, particularly in the terms of thermostability in the food production lines.

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