The influence of acid hydrolysis followed by autoclaving-cooling on physical properties and resistant starch of purple sweet potato (Ipomea batatas L.) flour

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Abstract. In this study influence of acid hydrolysis using HCl 2% followed by autoclaving-cooling at 121°C for 15 minutes and cooled at 5°C for 72 hours with two repetition cycles on resistant starch of purple sweet potato flour were investigated and physical properties were analyzed. This treatment exhibited beneficial impacts of modified flour on resistant starch formation by 14.35%. Significant difference was exhibited between native and modified flour for ΔE value. Rapid visco analyzer was also used to determine pasting properties including peak viscosity, trough viscosity, breakdown, final viscosity, setback, and peak time where all of them except breakdown value were higher than the native one. Moreover, no signs of damage on the surface of native purple sweet potato flour were showed in micrograph of scanning electron microscopy. While autoclaving-cooling sample exhibited a rough structure as a result of the loss of its granular appearance and destroyed shape. This study also indicated a change of X-ray diffraction pattern from C-type to B-type caused by the modification treatment.

Keywords: resistant starch, purple sweet potato, autoclaving, cooling

1. Introduction

Development of tubers-based modified flour including purple sweet potato recently have gained a great attentions. Those are triggered by several studies that reveal the functional properties of resistant starch contained in modified flour such as the prevention of diabetes risk, colon cancer, and acting as prebiotics [1,2,3]. Furthermore, the modification treatments also demonstrate the beneficial characteristics for food application such as viscosity and texture improvement as well as stability to temperature [4,5]. Purple sweet potato is a kind of prospective tubers to be improved as modified flour which is supported by the sweet potato productivity growth in Indonesia (168.18 ku / ha in 2016) [6].

Modification techniques actually can be performed either chemically, physically, or combination of those, nevertheless physical method is widely applied due to its ease and relatively low cost. Previous study on several kinds of starch (sago, dalugala/giant swamp taro, and taro) using heat moisture treatment (HMT) and autoclaving-cooling cycles (AC) method showed a rise of resistant starch statistically significant [7,8,9]. Autoclaving treatment causes amylase fraction to come out of the granules (gelatinization), while cooling causes amylose crystallization (retrogradation) [10,11]. Repeated AC treatment is able to improve the amylase-amylase and amylose-amylopectin rearranging thereby increasing the levels of resistant starch [11]. The increase of people’s attention to natural food products
that are free from chemical additives make those modification methods very prospective to be applied in purple sweet potato.

Lack of studies on the application of physical method on purple sweet potato flour encouraged this research, which aimed to evaluate the resistant starch content and physical properties changes of modified purple sweet potato flour caused by acid hydrolysis followed by physical methods, namely autoclaving-cooling with twice repetition cycles.

2. Experimental

2.1. Materials
Commercial purple sweet potato flour (Naya brand) purchased from local wholesale market in Bogor, Indonesia was used in the present study. HCl and NaOH were purchased from Merck (Germany).

2.2. Preparation of Acid Hydrolysis Followed by Autoclaving-Cooling
Acid hydrolysis of purple sweet potato followed by autoclaving-cooling used the method with slight modifications from Ozturk et al. (2011) [12]. 500 g flour was mixed with 2 L HCl 2%, then incubated at 40°C using shaker waterbath for 24 hours and neutralized with NaOH. After stored at 5°C for 24 hours, the flour suspension was dried using oven at 45°C, milled with a grinder and filtered using a 80 mesh. Subsequently, the hydrolyzed flour was added aquades at a ratio of 1: 4 for AC treatment at 121°C for 15 minutes and cooled at 5°C for 72 hours. The AC cycle was repeated twice, then oven dried at 45°C, grinded and sieved using a 100 mesh.

2.3. Resistant starch content
Resistant starch content was determined based on Goñi et al. (1996) [13]. The samples were incubated at 40°C and pH 1.5 for 60 minutes with pepsin (0.1 mL (10 mg/mL), Sigma) which was aimed to remove protein. Then, those were incubated with α-amylase (1 mL (40 mg/mL), Sigma) at 37°C and pH 6.9 for 16 hours which was objected to digest starch. The residue was treated with 2M KOH for solubilization of resistant starch that was subsequently incubated at 60 °C and pH 4.75 for 45 minutes using amyloglucosidase (80 mL (140 U/mL), Sigma) to hydrolyze the solubilized resistant starch. The glucose oxidase, peroxidase, and ABTS assay were used to determined free glucose [14]. The total starch was calculated as glucose × 0.9 which wheat starch (Sigma) was utilized as reference standard. The value of digestible starch was determined by the difference between total starch and resistant starch.

2.4. Color
The measurement of color was performed using NH310 colorimeter 3nh technology to obtain the hue angle and L*, a*, b* referring to CIE system, which the color coordinates range from L*= 0 (black) to L*= 100 (white), -a* (greenness) to +a* (redness), and -b* (blueness) to +b* (yellowness) were converted to color difference (ΔE). Colour difference was calculated using this equation:

\[
\Delta E = (\Delta L^*+ \Delta a^* + \Delta b^*)^{1/2}
\]

Where ΔL*, Δa* and Δb* are the colour parameter values of the difference between white standard plate (L*= 100.00, a*= 0.00 dan b*= 0.00) and colour values of the sample.

2.5. Pasting properties
The pasting properties of the starch were evaluated using Rapid Visco Analyser (RVA 4500, Perten Instruments, PerkinElmer Inc). 3 g flour sample was weighted in the RVA canister and was afterwards added 25 mL of distilled water. The following parameters including pasting temperature, peak viscosity, trough viscosity, breakdown, final viscosity and setback were recorded.

2.6. Scanning electron microscopy (SEM)
The morphology of the starch granules contained in the modified flour of purple sweet potato was observed using a scanning electron microscope (Shimadzu SSX-550). The samples were coated with
gold and confirmed by using SEM under an acceleration voltage of 15 kV, at a magnification of 250x, 1000x, and 3000x.

2.7. X-ray Diffraction (XRD)
Analysis of starch crystallinity type was performed by using XRD instrument Shimadzu XD610, Japan. A little amount of sample was located in the sample holder and exposed to the X-ray beam from the X-ray generator. Analysis was performed at 30 kV, 30 mA, and scanned in the 5-60° 2θ range.

2.8. Statistical analysis
Statistical Package for Social Scientists (SPSS, version 16.0) was used for statistical analysis. The results were evaluated by T-test at a significance level of p < 0.05.

3. Result and Discussion
3.1. Resistant starch content
Data in Figure (1) indicated that significant difference was found between the percentages of resistant starch of native purple sweet potato flour (13.70%) compared to modified flour using AC method (14.35%). That enhancement might be attributed by acid hydrolysis which was able to break down glycosidic linkage randomly both on amylose and amylopectin fraction. Furthermore, AC could cause not only gelatinization, but also retrogradation of amylose fraction where resistant starch level directly proportional to the amylose content of food [15,16]. Eerlingen et al. (1993) revealed that yields of resistant starch mainly depended on both storage time and on temperature where those influenced the kind of resistant starch crystallinity (A or B, X-ray diffraction pattern) [17]. In addition, amylose amylopectin ratio highly influenced retrogradation beside other factors such as the presence of foreign substances including sugars, salts, and emulsifier, molecular size, temperature, pH and other non starch components [18].

![Figure 1. Resistant starch content of native flour and modified flour. Data entries followed by different letters differ significantly (p < 0.05).](image)

3.2. Color
Color parameter is one of the most important sensory attributes affecting the consumer perception and preference in selecting food materials. Color parameters of native and modified flour obtained using CIE L*, a*, b* system (L*, a*, b*, hue and ΔE values) are summarized in Table 1. The native flour color visually showed a noticeable lighter color compared to modified flour (Figure 2). No significantly difference was observed between native (30.30) and modified flour (27.71) for hue parameter. The range of those values indicates that both samples are in the range of orange-brown color. While ΔE represents the change in level of intensity of colour of a sample with reference to a standard sample [19]. Significant difference was exhibited between samples for ΔE value. This could be attributed to the processing conditions involved in preparing the modified flour. The increased ΔE of modified flour leading to a
darkening color with might be due to presence of mono-and disaccharides in starch, which may have underwent caramelization and maillard reaction which involves the amino groups, proteins or any nitrogenous compound, and carbonyl groups of reducing sugars, aldehydes or ketones [20,21]. The reaction forming volatile (caramel aroma) and brown-coloured compounds (caramel colours) are leaded by the decomposition of sugars which can be effected by heat and is catalysed by acids and bases [22].

![Figure 2](image)

**Figure 2.** Visual appearance of native compared to modified flour.

| Sample | L* ± SE | a* ± SE | b* ± SE | Hue ± SE | ΔE ± SE |
|--------|---------|---------|---------|----------|---------|
| Native | 64.08 ± 0.37 a | 12.09 ± 0.10 a | 7.07 ± 0.15 a | 30.30 ± 0.41 a | 38.55 ± 0.38 a |
| AC     | 48.78 ± 0.71 b | 7.84 ± 0.82 b | 4.12 ± 0.52 b | 27.71 ± 0.58 b | 51.99 ± 0.54 b |

Data entries followed by different letters differ significantly (p < 0.05).

### 3.3. Pasting properties

RVA pasting properties of modified purple sweet potato flour and native flour are given in Table 2 and Figure 3. All RVA parameters, except breakdown value, of modified flour of purple sweet potato were higher than the native one. Native flour’s retrogradation degree was higher than native one which showed by setback value. Those phenomena were probably associated with the degradation of starch granules its solubility due to the effects of heat and pressure during preparation using autoclave [23]. The lower setback of the flours or starches, the lower tendency to retrograde [24].

![Figure 3](image)

**Figure 3.** The pasting curves of native flour and modified flour.

Due to low in viscosity, then this modified with higher resistant starch level could only be utilized as a filler or ingredient of food products as a source of resistant starch which has function like a food fiber in the body [9]. Peak time is the total amount of time required to achieve the peak viscosity. In other words, the time needed to totally gelatinize the starches in the flour samples. The presence of the components including fat, protein and other foreign components contained in the sweet potato flour could cause peak time alteration [25].
Table 2. RVA pasting properties of native flour and modified flour.

| Sample | Peak (cP) | Trough (cP) | Breakdown (cP) | Final Viscosity (cP) | Setback (cP) | Peak Time (minute) |
|--------|-----------|-------------|----------------|----------------------|-------------|-------------------|
| AC     | 477.00    | 278.00      | 199.00         | 510.00               | 232.00      | 3.20              |
| Native | 1147.00   | 998.00      | 149.00         | 1643.00              | 645.00      | 5.53              |

3.4. SEM micrograph

The SEM micrographs (Figure 4) of native compared to AC modified purple sweet potato flour are differed significantly. The micrographs exhibited that the native granules are tend to shape round with smooth surfaces and some granules were seemed oval. Furthermore, it had no signs of damage on the surface. While, the granular shape of modified flour was irregular after being acid hydrolyzed, two cycles of autoclaved-cooled and dried. AC sample showed a rough structure, towards to the loss of granular appearance and to a destroyed shape. Those phenomena were inferred that AC treatment where the temperature exceeded the gelatinization of starch, formed sponge like structure within the inner zone of the retrograded starch [26].

![SEM micrographs](image)

Figure 4. Scanning electron micrographs of native flour and modified flour.

3.5. X-ray Diffraction

The X-ray diffraction patterns of native and modified modified purple sweet potato flour are shown in Figure 5. Three kinds of X-ray diffraction pattern, depending on the organization of the double helix in the granules, are A, B, or C-type [27]. A-type starch is indicated by strong reflections at 20 angle consisted of about 15°, 23°, and an unresolved doublet at around 17° and 18°. B-type starch is exhibited by strong peak at about 17°, 5.6° as a characteristic peak, and several lower peaks at about 15°, 20°, 22°, and 24°, while C-type starch is a combination of both A and B-type [28]. AC sample exhibited a change of the X-ray diffraction pattern, compared to its native. Native purple sweet potato flour showed 20 peak intensities including at 14.97°, 15.01°, 17.93°, 17.97°, 23.1°, an unresolved doublet at 16.91° and 17.93°, and also stronger reflection intensity at angle 23.05°. After AC treatment, a change happened to the X-ray diffraction patterns of native sample with reflection intensities at 20 angle values of 14.9°, 17.01°, 19.7°, 22.06°, 23.8°, also greater reflection intensity at angle 16.97°. The sharper peak of modified flour diffraction pattern indicated the higher relative crystallinity which caused by retrogradation from AC treatment (Figure 5). The results showed that its crystalline pattern lead a mixture both A and B-type pattern, which could be classified as C-type and converted to B-type after AC treatment. It is in accordanadance with previous study that sweet potato starch which had doublet at 17.1° and 18.1° merged
into a greater peak at 17.1° after HMT treatment, and also the strong peaks of native starch at 15.5° and 24.8° were converted into shoulders, showing a change from C-type to B-type [29]. However, variable X-ray patterns could be affected by the different varieties of sweet potato starch [30,31,32].

![Figure 5. X-ray diffraction patterns of native flour and modified flour.](image)

4. Conclusion

The results showed that the modification pretreatment, namely acid hrydolysis, followed by autoclaving-cooling two cycles showed a beneficial impact on resistant starch formation compared to the native one. Hue values indicated that both native and modified flour had orange-brown in color. However, the modified one showed a darker color where its ΔE was significantly difference than the native flour. Compared to the native flour for the profil pasting pasrarameter, the preparation of modification obtained in the present study seem not to be suitable for the food products, however it could be utilized as a filler or ingredient as a source of resistant starch that has benefit as a food fiber alternative in the body. Furthermore, autoclaving at the beyond of gelatinization temperature followed by cooling cause loss of granular appearance which was exhibited by SEM micrographs and also change X-ray diffraction pattern from C-type to B-type. Further studies are required to achieve the best functional properties of modified flour preparations using autoclaving-cooling method.

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