Resistant starch content, pasting properties, and morphology of taro (Colocasia esculenta L. Schott) flour modified by heat-moisture treatment

Daniel Santoso, Ata Aditya Wardana* and Ingrid S. Surono

Food Technology Department, Faculty of Engineering, Bina Nusantara University, Jakarta, Indonesia 11480

*ata.wardana@binus.edu, ataaditya@gmail.com

Abstract. Taro flour was modified by heat-moisture treatment (HMT) in various times and aimed to evaluate its resistant starch content, pasting properties, and morphology. The moisture content of taro flour was adjusted into 25% before heating at 110°C for 4, 6 and 8 h to produce modified flour. HMT of 8 h and 6 h exhibited the similar amount of resistant starch compared to native flour, while HMT of 4 h resulted in significantly lower RS content. The modification process increased all of pasting parameters: peak, trough, breakdown, final viscosity, setback, peak time, and pasting temperature. No sign of fractures were exhibited on the surface of native taro flour granules visualized in SEM micrograph. While modified taro flour resulted in some cracks which might be due to partially gelatinized around the surface by heat and moisture treatment.

1. Introduction

Modified flour of taro will have higher resistant starch (RS) content, and have obtained a great attention since its beneficial effect to human health such as diabetic and colon cancer prevention due to prebiotic effect [1-3]. Moreover, higher RS content exhibits beneficial physical characteristics for foods especially its thermostability [4-5], and other properties such as its viscosity, resistant to shear, low pH [4]. Taro (Colocasia esculenta L. Schott) is a starch-rich tubers widely grown in Indonesia and potential to be modified in order to increase its RS content.

There are several modification methods in increasing the RS content, such as chemically, physically, or the combination. Physically modification are mostly by pre-gelatinization and heat-treatment method [6]. Repeated autoclaving-cooling and heat moisture treatment (HMT) have gained more attention to be implemented because of its simplicity to produce RS type III which is a result of retrogradation. It is also supported the people’s concern on natural and free from chemical additives based product. Furthermore, many consumers preferred HMT because it is considered safe and affordable. During HMT preparation, low moisture levels (<35%) starch granules were subjected the temperatures in the range of 84-130°C, above the glass transition temperature and below the gelatinization temperature, for certain period of time [7,8].

Limited information on the modified taro flour using physical method of HMT drived this study which aimed to evaluate the resistant starch content, pasting properties, and morphology of taro modified flour using HMT method.
2. Experimental

Materials
Commercial taro flour (Naya brand) was purchased from local wholesale market in Bogor, Indonesia was used in this study. Pepsin, α-amylase, KOH, and amyloglucosidase were obtained from Sigma-Aldrich (St. Louis, MA, USA).

Preparation of modified flour
HMT was used in this study by adopting the Collado et al. (2001) method with slight modification [9]. The moisture content of 200 g taro flour was adjusted to 27% by spraying the aquadest and mixed thoroughly. Then wrapped in aluminium foil and cooled at 4°C for 24 h for conditioning the sample. Furthermore, heated at 110 °C for 4, 6 and 8 h in an oven, followed by drying the flour using oven equipped by fan at 50°C for 4 h, and grounded, sieved at 60 mesh.

RS content
RS content was evaluated by adopting method of Goñi et al. (1996) [10]. Modified flour was added with pepsin (0.1 mL (10 mg/mL) and kept for 1 h at 40°C and pH 1.5, subsequently added with α-amylase (1 mL (40 mg/mL) and kept for 16 h at 37°C and pH 6.9, followed by residue treatment with 2M KOH. Then, it was incubated at 60°C, pH 4.75 for 45 min with addition of amyloglucosidase (80 mL (140 U/mL). The glucose oxidase, peroxidase, and ABTS assay were used to evaluate free glucose. Total starch was determined as glucose × 0.9 and wheat starch (Sigma) was used as reference standard. Digestible starch was calculated by the difference between total starch and resistant starch.

Pasting properties
Rapid Visco Analyser (RVA 4500, Perten Instruments, PerkinElmer Inc) was used to evaluate the pasting properties referring to the AACC Standard method No.61-02 (AACC, 2000) [11]. The parameters were recorded including pasting temperature, peak viscosity, trough viscosity, breakdown, final viscosity and setback.

Starch granule morphology
The morphology of the starch granules of the modified taro flour were scanned by using a scanning electron microscope (SEM) (Shimadzu SSX-550). Gold was used to coat the samples and then confirmed by using SEM. The instrument is set at voltage of 20 kV and at a magnification of 2500x, 5000x, and 10000x.

Statistical analysis
The results were statistically evaluated by analysis of variance (ANOVA) using Statistical Package for Social Scientists (SPSS, version 16.0) and followed by Duncan’s Multiple Range Test (DMRT) when needed at a significance level of p < 0.05.

3. Result and Discussion

Effect of HMT on the yield of RS
The RS yields of the samples treated by HMT at 110°C for 4, 6, and 8 h and the native flour are presented in Figure 1. The RS yield of the modified taro flour using HMT 4h (12.95%) is significantly (p < 0.05) lower than other modified taro flour samples (13.42% for HMT 6; 13.53% for HMT 8 h) and native (13.51%). This negative finding might be caused by the breakage of RS type 2 in the native flour as a result of heating process. RS type 2 might be hydrolyzed by enzymes and dissolved during sample preparation and RS analysis [12], in accordance with several studies reported the decrease of the total RS contents on HMT [13,14].
Figure 1. RS content of native and HMT flour. Data entries followed by different letters differ significantly (p < 0.05).

Compared to HMT 4 h, the higher RS contents of both HMT 6 and 8 h might be ascribed to the enhancement of amylose-amylose, amylopectin-amylopectin, amylose-amylopectin chains interaction in the amorphous region resulting more compact and stable structure [15]. Moreover, HMT allows the formation of new crystals and the formation of double helix among amylose molecules by hydrogen linkage which are more perfect during the modification process [16]. The lower RS of HMT 4 h might be associated to the loss of RS type II which tend to be more susceptible to heat compared to RS III.

Pasting properties
Table 1 and Figure 2 show RVA pasting properties of native and HMT flour suspension at various treatments. All pasting parameters of samples treated by HMT are higher than the native flour. Actually, that results are direct contrary to the expectation although other studies found the similar finding. Peak viscosity, trough and breakdown attribute of HMT samples are higher than native which indicate that their granules are more weak exposed by shear and heat, which is in agreement with previous study [17]. Final viscosity value indicates the paste stability to heat penetration in the usage which is usually in line with setback value which represents the starch undergoing retrogradation and syneresis [18]. In this study, HMT increase the value of both final viscosity and setback compared to the native.

Figure 2. Pasting properties of native and HMT taro flour

Pasting temperature represents the minimum temperature needed to start the gelatinization and also indicates energy cost. The peak time and pasting temperature of samples increase after HMT treatment. Those phenomena are possibly because of the enhancement of crystallinity degree. Earlier report stated that starch granules exposed by HMT need more heat penetration and time to disrupt its structure
followed by paste formation [19]. However, the longer time exposed by HMT led to lower all pasting parameters other than peak time and pasting temperature. Based on the pasting properties, particularly final viscosity which is the most broadly used to define a sample’s quality, those of modified flours are more appropriate as viscous pasting or gelling agent material.

| Sample | Peak (cP) | Trough (cP) | Breakdown (cP) | Final Viscosity (cP) | Setback (cP) | Peak Time (min) | Pasting Temperature (°C) |
|--------|-----------|-------------|----------------|---------------------|-------------|-----------------|------------------------|
| 8 h    | 966.00    | 638.00      | 328.00         | 1318.00             | 680.00      | 5.93            | 89.75                  |
| 6 h    | 2645.00   | 1981.00     | 664.00         | 3597.00             | 1616.00     | 5.93            | 86.35                  |
| 4 h    | 2837.00   | 2113.00     | 724.00         | 3840.00             | 1727.00     | 5.93            | 85.50                  |
| native | 911.00    | 614.00      | 297.00         | 1239.00             | 625.00      | 5.60            | 83.90                  |

**Starch granule morphology**

The morphology of the starch granules were observed using a SEM with magnification of 2500x and 10000x, showed in Figure 3. The HMT processing affects slightly the morphology of the starch granules of taro. The native starch has a smaller granule size than the modified ones, exhibited in Figure 3 with magnification of 10000x. Furthermore, some cracks are found on the modified starch which are presumed the effect of heat and moisture. The similar work using rice flour sample reported that some fractures of HMT starch granule surface were caused by swelling or partially gelatinization around the surface [20].

**Figure 3.** Starch granule morphology of native and HMT flours at 2500x and 10000x magnifications.

4. **Conclusion**

The holding time of HMT modification affected RS content, pasting properties, and starch granule morphology of taro flour. However, using HMT for 4 h decreased RS yield compared to native due to heating process, which generating the breakage of RS type 2 in the native flour. Furthermore, all of pasting characteristics of HMT flours shifted to the higher temperature. Also, the HMT processing resulted an alteration to the starch granule morphologies. Based on the result in the present study, it seems only suitable for the certain food products, but inappropriate as a beneficial source of food fiber in the form resistant starch. However, it need further study regarding modified taro flour thermostability during cooking.
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