Chromatographic profiling to determine the extent of structural changes in arrowroot starch (*Marantha arundinacea* L.) modification

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**Abstract**

Arrowroot starch (*Maranatha arundinacea* L.) has demonstrated to have a potential resistant starch type III (RS3). Certain techniques of starch modification could increase the yield of resistant starch. The effect of multiple treatments of acid hydrolysis, debranching, autoclaving-cooling cycles, and heat moisture treatment (HMT) towards the structural changes of arrowroot starch, especially with HMT as the additional treatment, was investigated in this study. This study revealed the molecular distribution profile of arrowroot starch after some modification, which might be related to the starch resistance, by using gel filtration chromatography (GFC).

GFC profiles could distinguish the extent of starch structural changes, shown by the different molecular distribution profile. In general, modification by the multiple treatments led to the decrease of amylopectin and increase of amylose fractions. Amylose fraction has been known to contribute to a more rapid retrogradation process as a mechanism of resistant starch formation. Combination of acid hydrolysis, debranching, autoclaving-cooling, and HMT treatments obviously altered the starch structures according to GFC profiles. The intensive degradation of amylopectin into amylose fragments could be determined. This fact might be associated to the starch resistance.

**Introduction**

Resistant starch (RS) has become an interest for many researchers not only due to its benefit for health but also its functional properties for food processing (Sajilata et al., 2006; Milasinovic et al., 2010). Resistant starch refers to the portion of starch and starch products that resist digestion as they pass through the gastrointestinal tract (Fuentes-Zaragoza, et al., 2010).

Arrowroot starch (*Maranatha arundinacea* L.) has the characteristics which suits the requirements for producing resistant starch type 3 (RS3) (Wang et al., 1998; Srichuwong et al., 2005). Some methods of starch modification have been known to increase the resistant starch. Multiple treatments of acid hydrolysis, debranching, autoclaving-cooling, and particularly in combination with heat moisture treatment (HMT) that subjected towards arrowroot starch have not been much explored in other studies. In this research, Gel Filtration Chromatography (GFC) technique was used to determine the effect of the given combination of treatments to the starch structure of arrowroot starch. Gel Filtration Chromatography (GFC) is a mode of liquid chromatography in which components of mixture are separated on the basis of size. In GFC, large molecules elute from the column first, followed by smaller molecules. The molecular distribution of various native and modified or treated starches have been determined by using GFC technique (Lu et al., 1996; John et al., 2002; Ferrini et al., 2008; Ozturk et al., 2011). The technique can quantitatively separate starch using molecular weight distribution and mean degree polymerization (Lu et al., 1996). This research was aimed to determine the changes of molecular distribution profile by gel filtration chromatography as a result of arrowroot starch modification, subjected to the characteristic of resistant starch formation.

**Materials and Methods**

**Modification of arrowroot starch**

Arrowroot starch (*Maranatha arundinacea* L.) was obtained from Woman Farmer Association (Yogyakarta, Indonesia). The procedures of acid hydrolysis followed the method by Aparicio-Saguilan et al. (2005), debranching treatment followed the method of Zhao and Lin (2009) with a modification employed by doing the pre-heating of suspended starch at 80°C for 5 mins before conducting the autoclaving step at 121°C and the use of pullulanase enzyme (Sigma P-2986, Sigma-Aldrich, USA) from *Klebsiella pneumoniae* at 10.4 U/g, autoclaving-cooling treatment followed the method of Lehmann.
et al. (2003), while procedure for HMT was adapted from Chung et al. (2009), with modification (20% moisture, 121°C, 15 min).

Profile of starch molecular distribution

The profiles of starch molecular distribution were determined by using GFC columns packed with Sephacryl S-400 HR gel (2.6 cm i.d. x 17 cm) (Sigma, Sigma-Aldrich, USA) and Sephadex G-50 superfine (2.6 cm i.d. x 95 cm) (Sigma, Sigma-Aldrich, USA). Sephadex G-50 gel (1.5-30 kDa) was used for enzymatic modified samples (DE-AC, DE-AC-HMT, AH-DE-AC, AH-DE-AC-HMT) (John et al., 2002). Sephacryl S-400 HR (20-8000kDa) was used for non-enzymatic modified samples (native, AC-HMT, AH, AH-AC-HMT) (Rodis et al., 1993). Fat-free sample (20mg) was suspended with 10 mL distilled water, autoclaved at 105°C for 1 hr, and centrifuged (4°C, 1000 g, 10 mins). The supernatant (2.0 mg/mL) was applied into GFC column (4 mL and 7.5 mL of sample for sephacryl and sephadex, respectively). A solution made of distilled water containing 25 mM NaCl and 1 mM NaOH was used as an eluent. Fractions of defined volume (5.0 mL and 10.0 mL of eluate for sephacryl and sephadex, respectively) were collected by using fraction collector model SF-100 (Toyo, Toyo Kagaku Sangyo Co., Japan) and analyzed for total carbohydrate (phenol-sulfuric acid method) (Dubois et al., 1956) and blue value (iodine staining) at 490 and 630 nm (Juliano, 1971), respectively.

Principle Component Analysis (PCA) by using XLSTAT 2017 software was used to analyze the region division in chromatogram as a plot between tube/fraction number and absorbance (of total carbohydrate and blue value analysis). The output of PCA is a scoreplot which could be used to recognize the pattern in order to obtain the region division in the chromatogram.

Results and Discussion

GFC elution profiles of native and non-enzymatic modified samples obtained by using Sephacryl S-HR 400 are shown in Figure 1. In general, all samples were separated into two dominant distribution area by looking at total carbohydrate profile (Figure 1 A-D). The first peak was generally considered as amylopectin, having higher molecular mass, indicated by the higher peak in total carbohydrate profiles but this peak had a lower peak by looking at blue value profiles. Thus, the second peak, with strong blue value response, corresponded to amylose.

The elution profile of native showed a strong response of amylopectin, but relatively weak response of amylose. In AC-HMT sample, the amylopectin fraction was found to decrease, which caused by thermal degradation of amylopectin during autoclaving-cooling and HMT, while the amylose fraction was observed to increase. The increased amount of amylose after autoclaving-cooling cycles was associated with the increased yield of resistant starch (Sievert et al., 1989). This was because amylose tends to retrograde more rapidly than amylopectin. Retrogradation has been known as a mechanism of resistant starch formation. Thermal degradation of amylopectin in HMT-treated starches was also revealed in other studies by analysis with GFC (Lu et al., 1996; Li et al., 2010). A correlation exists between amylopectin content of the starches and the yield of resistant starch was reported in several studies (Sievert et al., 1989; Shu et al., 2007; Zhu et al., 2011; Mir et al., 2013).

In AH sample, the first peak corresponded to amylopectin which had not been hydrolyzed by acid. The second peak corresponded to amylopectin fraction which had been hydrolyzed by acid. The amylopectin was lower compared to native and AC-HMT samples, suggesting the more degradation of amylopectin. The third peak was considered as amylose fraction, indicated by the higher peak in blue value profile. The amylose fraction of AH was higher compared to that of native and AC-HMT, indicating higher amount of amylose fraction generated after acid hydrolysis. The degradation of amylopectin as the impact of acid hydrolysis was also reported in

Figure 1. GFC profiles of arrowroot starch using Sephacryl S-HR 400 column (2.6 cm i.d. x 17 cm) and solution of 25 mM NaCl and 1 mM NaOH in water as an eluent: (A) native; (B) AC-HMT; (C) AH; (D) AH-AC-HMT.
Other studies (John et al., 2002; Mun et al., 2005; Ozturk et al., 2011).

In AH-AC-HMT the amylopectin was not identified anymore. The first peak corresponded to amylose, indicated by the strong blue value response. The second peak was considered amylose with lower molecular mass, as HMT treatment might break down the amylose into shorter chain of amylose. HMT has been showed to degrade linear chain of starch into the shorter chain (Lu et al., 1996), which presumed to be occurred in this study.

GFC elution profiles of enzymatic modified samples obtained by using Sephadex G-50 are presented in Figure 2. In DE-AC, the first peak was considered amylose fraction, indicated by the strong blue value response. The second peak was considered a shorter chain of amylose fraction with lower molecular mass. The evidence showed that the debranching treatment had successfully broken down the α-1,6 branch points of amylopectin, generating the short chain amylose. The results were concomitant with the results reported in debranching of corn, arrowroot, and sago starch (Han et al., 2003; Leong et al., 2007).

In DE-AC-HMT sample, the GFC profile did not show a significant change of total carbohydrate profile, compared to that of DE-AC. However, the blue value profile showed a very weak response. The thermal exposure given during HMT treatment could break down the amylose into shorter chain of amylose which might not be able to form a complex with iodine anymore. The decrease of blue value response as the impact of HMT was also reported by other author (Sair, 1967). The observed evidence could also be explained by complex formation of amylose which was triggered by high temperature applied during HMT, leading to the decrease of iodine-binding capacity of amylose (Bhatnagar and Hanna, 1994).

Profile of AH-DE-AC also showed that the first peak corresponded to amylose. The amylose of AH-DE-AC appeared as a broader peak, compared to that of DE-AC. This evidence indicated that the AH treatment which was applied before DE-AC caused the more heterogeneity of crystals. The increased heterogeneity of crystals after acid hydrolysis was also reported in cassava and maize starch (Ferrini et al., 2008). The second peak corresponded to amylose with smaller molecular size.

The two dominant distribution areas which found in other samples disappeared in AH-DE-AC-HMT. This indicated that the combination of treatments caused relatively more intense degradation of amylopectin molecules. While blue value analysis showed a very weak response that could be explained with the same reason for DE-AC-HMT. The sample of AH-DE-AC-HMT showed relatively the most intense degradation of amylopectin which might suggest the more possibility of resistant starch formation during retrogradation.

The validity of region division in chromatogram of the eight samples (showed in Figures 1 and 2) was confirmed by using Principle Component Analysis (PCA) which could be explained by 100% principle component (total PC1 and PC2). Figure 3 showed the representative of scoreplot result obtained in the PCA analysis of AH sample. It could be seen from the figure that the fraction division in chromatogram (presented...
Conclusion

Chromatographic profiling with GFC showed that the different combination of treatments applied in arrowroot starch led to different extent of structural changes of the starch. However, generally the treatments caused the increase of amylose fraction and the decrease of amyllopectin fraction. The increased amylose fraction might promote the possibility of resistant starch formation through retrogradation. Profiling with GFC is therefore a useful technique to observe the structural changes of starch which might be related to the starch resistance.

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References


