Characterization of Type-III Resistant Starch Produced by High Shear Mixing Combined with Membrane Separation

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***Abstract*— There are five types of resistant starch and the most commonly used as functional food is type-III resistant starch (RS-III). RS-III is a retrograded of gelatinized starch that is conventionally produced by heating and cooling treatment. This study characterized the RS-III produced by an unconventional method by modification of high shear mixing (HSM) combined with membrane microfiltration from cassava starch. In this research, a starch/water mixture with a concentration of 1/20 w/v was gelatinized in the HSM reactor at 95°C for 15 minutes and then separated using membrane microfiltration. The separated permeate was cooled to the retrogradation process. The products were characterized by iodine, X-ray diffraction (XRD), Scanning Electron Microscopy (SEM), and Total Dietary Fiber (TDF) content. From the experiment, it was found that the higher HSM speed significantly increased the TDF content up to 12.33% and decreased the amylose content and crystallinity of the RS-III products.**

***Keywords*—Amylose, Cassava starch, High shear mixing, Microfiltration, Resistant starch type-III**

I. Introduction

Deadly global epidemics such as diabetes mellitus keep increasing even in this health-conscious era. The symptom of diabetes is exceeding blood sugar levels that cause abnormality of insulin metabolism [1]. Data from International Diabetes Federation stated that in 2021 Indonesia has 19.46 million diabetes cases with a productive age range [2]. It is very crucial to find solutions to prevent this number to increase or even better if it can be cut down. One way to achieve this is to consume a healthier diet.

The way to eat healthy food is usually achieved by consuming functional food that has several or specific benefits for human health. One example of this functional food is resistant starch. Resistant starch (RS) is defined as a fraction of starch that resists the digestive enzyme and is fermented by the colon microbe [3-4]. There are five types of RS, type-I is the indigestible and physically trapped starch, type-II is the ungelatinized starch that naturally resists the digestive enzyme, type-III is the retrograded starch from gelatinized starch, type-IV is the chemically-modified starch [5-6], and type-V is the resistant starch that formed by complexation of amylose-lipid [7].

The RS type-III (RS-III) is greatly getting attention for functional foods future study. It can be produced from cassava starch. Starch from cassava has amylopectin and amylose with percentages of 80-83% and 17-20%, respectively [8]. Amylopectin is a highly branched polymer and amylose consists mostly of linear polymer, which in the granules these two polymers construct semi-crystalline structures [9]. The double helices of amylopectin dominated the crystalline region while amylose formed amorphous region interspersed with the branched segment of amylopectin [10].

The traditional way to obtain RS-III from starch is conducted with the heating-cooling cycle. When heated in excess water, the starch granules are disrupted and start to swell because of the water absorption. Amylose in the granules will leach out from the granules as a random coil and resulting in increased viscosity. This stage is called the gelatinization process [11-12]. Then these disrupted granule molecules will gradually slow down and rearrange into more ordered structures within cooling. And this stage is called retrogradation [13].

The conventional way to obtain resistant starch are heating-cooling cycles, enzymatic debranching, extrusion, and microwave [14-18]. These conventional methods are usually done on high-amylose starch, whereas in this study, we used cassava starch that has low-amylose content and high amylopectin content. The presence of amylopectin has a negative impact on resistant starch production by binding the amylose in its structure and also disrupting the reassociation of amylose. High shear mixing (HSM) can be used to enhance the gelatinization process. HSM is a set of rotor-stator systems operated in high speed that generated high shear force. HSM is equipped by centrifugal rotor that installed in a stator with holes which could generate very high-speed rotation (3,600-10,000 RPM). The comparison using this kind of mixer than the conventional for batch mixing is the materials are moved through a workhead so the mixing can be optimized. This shear force exhibited by HSM effectively degrade the starch mechanically and even homogenized starch-water mixture [19-21]. Previous study used HSM to degrade the starch granules and resulting in increasing viscosity-average molecular weight that implied the HSM mostly remove amylose [22].

During gelatinization hydrogen bonds of the starch granules continuously broken and reformed through the increasing temperature [15]. Most study found that the starch started to gelatinize at temperature of 66.8-77.12°C [19-20]. Han et al. reported that amylose leaching

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increased as the higher degree of granules swelling as the temperature increased for the same time [25].

The idea to separate leached amylose from the gelatinized starch can be done using membrane separation based on molecular weight. Membrane is a barrier that can separate a specific component and retain another component from the same fluid stream [26].

The microfiltration membrane has 0.1-10 µm pore size that can be used to separate amylose with molecular weight of $1.5x10^5$ [27-28]. The amylose separation aimed to yield higher RS-III products because many research proved that RS-III retrogradation is proportional to amylose content [29]. A research found that amylose can be separated from granules using microfiltration membrane for maize starch [30].

This research investigated the characterization of the RS-III produced by combining HSM with microfiltration. The aim was to enhance the amylose leaching by HSM and increase amylose separation by a membrane to increase the resistant starch formed in the retrogradation process. The characterizations included the total dietary fiber (TDF) content, amylose content, X-ray diffraction analysis, and scanning electron microscopy (SEM).

II. Method

A. Materials

The starch suspension with a ratio of 1/20 g/mL was used in this study using commercial tapioca flour (PT Sungai Budi, Jakarta, Indonesia). The membrane used for the filtration process was a polypropylene membrane with a 10µm pore size and an effective area 10cm x 3.5cm (Hangzhou Tianshan Precision Filter Material Co., Ltd, Hangzhou, China).

B. Methods

The starch suspension was made to high shear mixing (HSM) reactor (Figure 1) with volume of 200mL. The HSM speed variables used were 3,000; 6,000; and 9,000 RPM at 95°C for 15 minutes. Oil-bath system was used for the heating media and cooling water was used to reduce the heat on the rotor-stator bearing seal.

The hot products from HSM were diluted with 300mL of distilled water and let to cool down to 40°C to make 500 mL feed for the microfiltration. Dead-end configuration was used in the membrane microfiltration with a pore size of 10µm. The permeate was then cooled in the refrigerator at ± 4°C for 24 hours to start the retrogradation process for RS-III formation. The final products were dried using a freeze-drier before characterization.

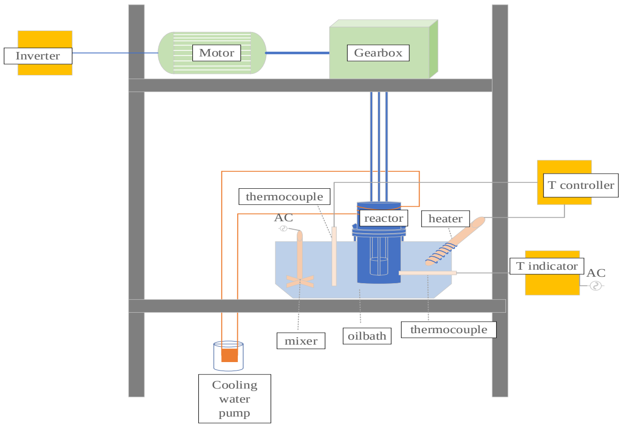

Figure 1. Experimental setup of high shear mixer apparatus

The effect of the HSM speed variables was characterized with several analysis. The analysis of total dietary fiber (TDF) content to quantify the amount resistant starch of the products, amylose content analysis, relative crystallinity degree to calculate the change of crystallinity of the RS-III products and the morphological structure change of the products.

C. Analysis for Characterization

1) Total Dietary Fiber (TDF) Content Analysis

The total dietary fiber (TDF) content of the products was analyzed using AOAC official methods 993.19 and 991.42 for dietary fiber measurements.

2) Amylose Content Analysis

Spectrophotometer UV-Vis was used to analyzed the amylose content of the products with iodine method based on Zhu et al. (2008) [13],[31].

3) Relative Crystallinity Degree (RCD) Analysis

The crystallinity was investigated at Department of Materials and Metallurgical Engineering, Institute of Technology Sepuluh Nopember Surabaya, Indonesia using X-ray diffraction (XRD) equipment XRD Philips Xpert MPD with a diffraction angle of 2θ and range from 0° to 40°. The relative crystallinity degree (RCD) was calculated using the software Origin2021 by the following equation:

$$RCD = (\text{crystalline area} / \text{total area}) \times 100\% \quad (1)$$

4) Scanning Electron Microscopy (SEM) Analysis

The granules morphology was observed at Mechanical Engineering Department Institute of Technology Sepuluh Nopember Surabaya, Indonesia using a Zeiss Evo MA-10 (Jena, Germany) with a magnification factor of 100 for products and 1,000 for native.

The average particle diameter was calculated using ImageJ software by measuring the average area of the particles.

III. Results and Discussion

The effect of combination of high shear mixing (HSM) and membrane microfiltration on resistant starch type-III (RS-III) products was investigated. The formation of RS-III conventionally occurs as the leached amylose from gelatinized starch start to recrystallize and form new hydrogen bonds that resist the digestive enzyme when the starch is cooled down and undergoes a retrogradation process. This conventional method is usually done on high-amylose starch that can form a higher RS-III yield. But this study used low-amylose starch so the conventional method could not reach the high RS-III yield.

The quantity of resistant starch was analyzed using total dietary fiber (TDF) content analysis. This is the commonly used method to estimate the resistant starch content in food products [32]. This method is an in-vitro enzyme-gravimetry that represents resistant starch values which is an undigested starch by the digestive enzyme [33]. As shown in Table 1, the native starch used in this study had a TDF content of 0.24%. As the speed of HSM increased to 3,000; 6,000; and 9,000 RPM, the TDF content of the products also increased become 5.78%, 9.10%, and 12.33%, respectively as shown in Table 1.

TABLE 1.
TOTAL DIETARY FIBER (TDF) CONTENT ANALYSIS OF RS-III PRODUCTS

HSM Speed (RPM)	TDF Content (%)
0 (Native)	0.24
3000	5.78
6000	9.10
9000	12.33

Increasing TDF content in the products represented that the gelatinization enhanced by HSM and combined with membrane microfiltration could increase the enzyme-resistant bonds formation. The shear force generated by HSM at 3,000-9,000 RPM could disrupt the starch granules in the gelatinization process which was also accompanied by the heating at 95°C causing more disruption and dissolution of the starch granules. On the other hand, the previous study by Sumarno et al. (2023) used HSM to produce RS-III and reached a maximum TDF content of 3.67% with 1/7 w/v concentration at 6,000 RPM [34]. This implied that the leached amylose separation held an important factor in the TDF increment in the products.

However, the TDF content of the products produced by the proposed method still could not reach the commercial products TDF or RS content. The TDF content of commercial products such as Crystalean, Novelose330 (Ingredion Inc.), C* Actistar (Cargill), or Neo-Amylose that have TDF content of 19,20%; 30%, 53%, and 87%, respectively [14].

The amylose from cassava starch has a molecular weight of 2.4-2.7 x 10^5-10^6 Da, meanwhile, amylopectin has a molecular weight of 5-40 x 10^6-10^8 Da [35]. With the membrane used in this study having a pore size of 10μm (MWCO ~ 10^7 Da), the amylose should mainly pass through the membrane pore, and amylopectin was mainly retained on the membrane. However, from the experiment, all the products could flow through the membrane which mean that the high molecular weight of amylopectin was broken down into smaller molecular size that could pass the membrane pores.

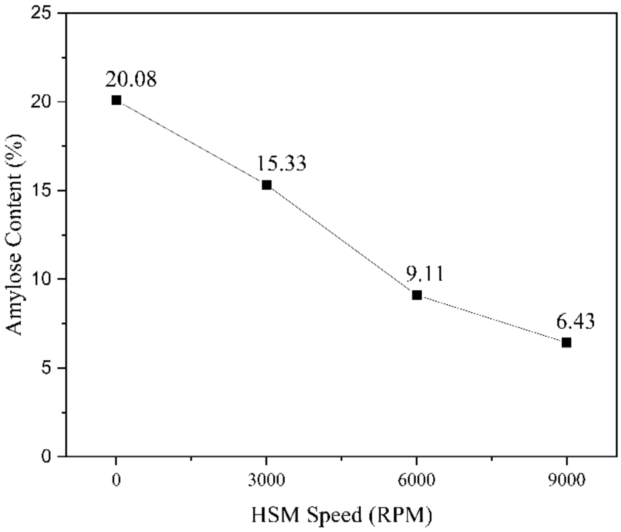

Figure 2. Amylose content of resistant starch type-III produced by combination of high shear mixing (HSM) and membrane microfiltration

Amylose content in the starch-based products has a positive effect on the RS-III formation. The higher the amylose content of the products, the higher the RS-III yield because the leached amylose from starch granules formed RS-III faster than amylopectin in the retrogradation process [36].

The amylose content of the RS-III produced in this study was analyzed using the iodine method with modifications based on Zhu et al. (2008) [31]. The native cassava starch used in this study had amylose content of 20.08%. As shown in Figure 2, the amylose content was decreased to 6.43% as the speed of HSM increased up to 9,000 RPM. The higher the speed of HSM would make higher shear generated and higher disruption of the granules. But this high shear force could also damage the granules and made the amylose from granules could not be detected as amylose by iodine binding.

The finding in this study might decline the claim that high RS is associated with high amylose content. The amylose content of this research is reduced while the TDF content increased. This could be caused by the formation of retrograded amylose that formed resistant bonds that resist the digestive enzyme. Another speculation also rised from this phenomenon that the resistant starch formed from this study was not detected as amylose in the iodine method of amylose analysis. This could explain the increasing TDF content of the products while the amylose content decreased.

The granules disruption from the shear force also proven by the reduced of relative crystallinity degree (RCD) of the RS-III products. The RCD was analyzed by X-Ray Diffraction (XRD) to identify the crystalline structure of the products. As shown in Figure 3, the native cassava starch used in this study had a semi-crystalline diffractogram with strong peaks at 15°, 17°, 18° dan 22,9°. These peaks showed that the native had type-A polymorph diffraction [37].

The RCD of native starch was 38.87% and with increasing HSM speed variables up to 9,000 RPM, the RCD of the products significantly reduced to 7.78%. This phenomenon is presumed due to the shear force generated by HSM greatly affected the crystalline region of the granule that made the products mostly become amorphous. Figure 2 also showed that the crystalline peaks disappeared from the polymorph diffraction that made the crystallinity structure of the products was amorphous.

The product with the highest TDF content had reduced RCD. The factor of high amylopectin starch are more sensitive to shear stress under constant pressure also become consideration [16],[38]. Arp et al. also found reduced crystallinity of RS products using thermal treatment [39]. Other researcher found that the enzymatic resistance to the specific product could not be estimated by the crystalline type of the starch products [40].

The ratio of starch/water might also affect the reduced crystallinity. This study used ratio of 1/20 g/mL which had ~95% water. The excess water could inhibited the recrystallization process and only amorphous aggregates could form in the retrogradation process. Zhou et al. (2011) found there were no amylopectin chains crystallization at 80-90% water content due to water molecule was in between of the chains so could not form the crystal structures [41].

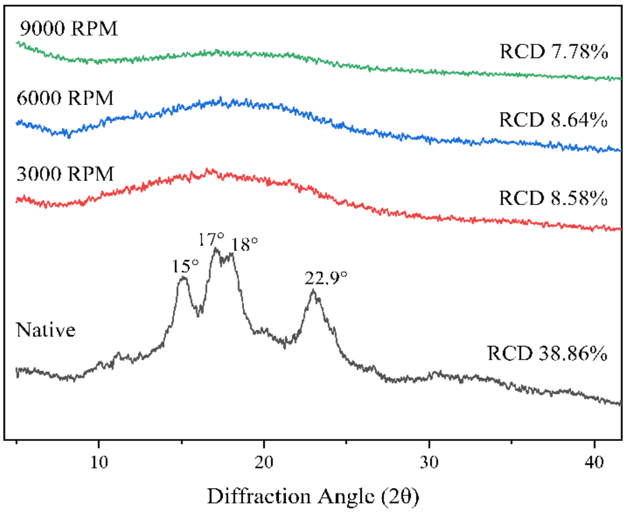

Figure 3. X-ray diffraction (XRD) pattern of resistant starch type-III produced by combination of high shear mixing (HSM) and membrane microfiltration

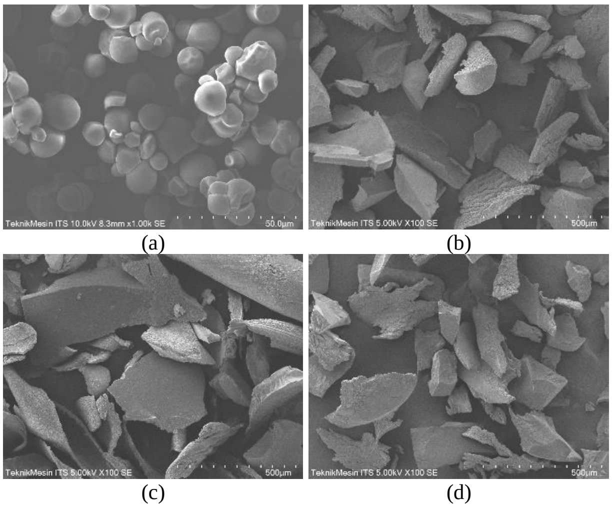

Figure 4. Morphology of resistant starch type-III produced by combination of high shear mixing (HSM) and membrane microfiltration by scanning electron microscopy (SEM). a) native starch with mag. 1000x; b) variable 3000 RPM with mag. 100x; c) variable 6000 RPM with mag. 100x; d) variable 9000 RPM with mag. 100x

Morphology characteristic of the RS-III products was analyzed by scanning electron microscopy (SEM). The native cassava starch granules have smooth surface and dominantly spherical-shaped. The image also showed the granules were close and adjacent to each other.

After processed with high shear mixing and microfiltration, the granules underwent a retrogradation process that made it possible to form a new shape that were dominantly irregular-shape as shown in Figure 4. This explained that HSM disrupted the structure of native starch caused by shear force imposed to the granules. The granules became flat-thin and had rough surface, it showed that the granules of the products lost its natural granule characteristics.

TABLE 2.
AVERAGE PARTICLE DIAMETER OF RS-III PRODUCTS

HSM Speed (RPM)	Average Particle Diameter (mm)
0 (Native)	0.023
3000	0.271
6000	0.267
9000	0.258

Table 2 showed the average particle diameter of the RS-III products. The native starch has average particle diameter of 0.023 mm. After processed using high shear mixing and microfiltration, the average particle diameter of the products increased up to ten times larger than the native starch average particle diameter. There were no significant differences with the increasing the HSM speed variables. This larger particle size also supported the higher value of total dietary fiber of the products because larger particle size tends to take more time to be digested by digestive enzymes.

Wang et al. also found increasing particle size distribution due to the water absorption that caused granules swelling and formation of large and dense amorphous structure during retrogradation [42]. The slower enzymatic hydrolysis was reported by De La Hera (2013) for the longer size of rice starch particle. This is due to the decreasing particle size create more exposed area to the digestive enzymes and leading to increasing rate of digestion [43].

IV. CONCLUSION

This study investigated the total dietary fiber content, amylose content, relative crystallinity degree and morphological structure of resistant starch type-III produced by combination of high shear mixing (HSM) and microfiltration. From the experiment, it was found that the higher HSM speed significantly increased the TDF content up to 12.33%. The products resulted in decreasing the amylose content. The relative crystallinity of the products was decreased to become mostly amorphous. The products also lost their native granules morphological characteristics to become dominantly irregular-shape and had rough surface. The results of this study showed that the method of combining HSM and microfiltration could increase the RS-III production.

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