

Improving the resistance starch of rice through physical and enzymatic process

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Abstract. People with obesity and diabetes mellitus in Indonesia have increased in number from year to year. One of the reasons is caused by high glycemic index (GI) value in rice as our staple food. The GI of the rice ranged from 64 ± 9 to 93 ± 11 , where glucose = 100. The objective of this research is to compare several processes in decreasing the GI value of rice, i.e. acid hydrolysis; acid hydrolysis followed by autoclaving-cooling and hydrolysis by pullulanase enzyme. GI values in this study was determined through the percentage of the resistant starch (%RS) as the opposite of GI. The experiment was started by analyzing the viscosity and gelatinization temperature, moisture content, resistant starch, and carbohydrate content of the rice starch. Variations used in this experiment are the type of acid solution (hydrochloric acid, acetic acid, citric acid, and lactic acid), acid concentration (0.01, 0.05, 0.1, 0.15 and 0.2 mole / L), and period of pullulanase hydrolysis (2, 6, and 8 hours). Condition of heating - cooling used was 121°C and 4°C. The results showed that the highest resistance starch content (7.6%) is obtained from the hydrolysis process using pullulanase enzyme for 8 hours.

1 Introduction

Obesity and diabetes mellitus have become a global health problem in developed and developing countries. According to the survey conducted by WHO in 2003, it was showed that 17.5% of Indonesia's population classified as overweight and 4.7% obese. By 2015, WHO estimated that 2.3 billion adults in the world were overweight and 700 million were obese.

Referring to those matters, nowadays, food should meet not only nutritional value, but also functional value in maintaining the health. The study on this subject has increased along with the increasing awareness of the importance of healthy living.

One of the main things that are important in contributing human health is the right choice of food. Choose the right food can be done through glycemic index (GI) approach. This concept is helpful in identifying carbohydrates based on their effects on blood sugar levels and insulin response. Carbohydrates with low glycemic index value indicates the decomposition level of carbohydrates into glucose in small intestine. The lower GI, the increasing glucose and insulin in blood will be more slowly and gradually. This is important for diabetics in controlling their excessive blood sugar levels.

Starch as the main carbohydrate in the plant consists primarily of D-glucopyranose polymers linked together by glycosidic bonds (α -1, 4 and α -1, 6). Polymerization of glucose in the starch produces 2 types of polymers, i.e. amylose and amylopectin. Amylose is a linear polymer which is mostly composed of α -1, 4-linked D-

glucopyranose. Whereas, amylopectin is the dominant molecules in most normal starches, larger and branched molecules of amylose. Amylopectin is composed of α -1, 4-linked D-glucopyranose connected by α -1, 6-linked branch point [1].

Starch also has a unique characteristics in the presence of water and heat, such as gelatinization and retrogradation. Retrogradation and gelatinization process is the most important part that can affect some changes on starch characteristics. Gelatinization is the process in which water enters the starch granules so that the granule swells and eventually ruptures. This process takes place between the temperature of 60-70 °C, but generally varies for each type of starch. After no heating anymore, the polymer chains (amylose and amylopectin) of starch will rearrange their structure, which is usually called as a retrogradation process. Retrogradation is a process of rearrangement of starch component to form a crystal.

Until about 15 years ago, starch is considered to be completely digested. However, in 1982, Dr. Englyst introduced the term of "resistant starch". It means the starch that cannot be hydrolyzed by incubation with α -amylase and pullulanase during determination of non-starch polysaccharides [2]. Resistant starch is a fraction of starch remaining undigested by digestive enzymes in small intestine of healthy human. Therefore, it has a nutritional implications in food because it causes a low rate of starch hydrolysis in the digestive tract. Foods that contain resistant starch, can be used to control blood sugar rise and reduce the risk of diabetes mellitus.

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Several methods have been carried out to form resistant starch and produce a significant yield. One of them, reported by the Edmonton and Saskaton [3] that the resistant starch content can be increased by repeat the heating and cooling of gelatinized starch. In this research to reduce the cycles, the starch is hydrolyzed prior heating and cooling. In addition, starch resistance can also be increased by enzymatic process. The starch was hydrolyzed using a pullulanase enzyme [4,5]. Debranching by a pullulanase enzyme can produce a linear and low molecular weight polymer. Then, this polymer chain can be recrystallized and increase the possibility of aggregation molecules.

Ranawan et al. [6] in their study on the commercially available rice and rice products found that their GI are ± 90 . Since our staple food is rice, so the decrease of its GI is necessary in terms of the population health. So, the goal of this research is to improve the GI of rice through physical and enzymatic process.

2 Material and methods

2.1 Materials and reagents

As a raw material, it was used a "Slyp Sari Rasa" broken rice from Cianjur and Pullulanase enzyme (Promozyme® D2, with enzyme activity 1584.3 NPUN/ml) obtained from Novozyme, Indonesia.

2.2 Experimental set-up

Rice used in the form of flour which was prepared by milling the granules and drying in the oven at temperature of $\pm 35-40$ °C. Then the viscosity and gelatinization temperature of rice flour were determined using a Brookfield Rheometer; the resistance starch content was analyzed by Gravimetric Enzymatic method (AOAC 1995), and the carbohydrate content by Direct Acid Hydrolysis method (AOAC 1970).

The variables used in this research were the type of acid solution i.e. hydrochloric acid, acetic acid, citric acid, and lactic acid; the concentrations of acid solution i.e. 0.01, 0.05, 0.1, 0.15 and 0.2 M, and the pullulanase hydrolysis time i.e. 2, 6, and 8 hour).

This research was conducted in three variations of the process i.e. (1) the acid hydrolysis of rice flour, (2) the result from (1) continued by heating-cooling (autoclaving-cooling) and (3) the hydrolysis with pullulanase enzyme. Finally, the product was determined its resistant starch content (% RS).

In the hydrolysis with acid, the acid solution used were varied in type and concentration. It was added into the rice flour at the temperature 35°C for 8 hours. Then, adjustment of pH was performed with the addition of NaOH up to pH 6. Furthermore, the suspension was centrifuged with rotational speed of 5000 rpm for 10 minutes. The residue obtained being washed with distilled water, dried in the oven at 50 °C, milled, sifted, and analyzed for its %RS.

All the flour obtained from the hydrolysis with acid were autoclaved and cooled. Firstly flour was suspended in water (40% w/v). Then the suspension was heated and stirred until homogeneous and viscous before it was autoclaved at 121 °C for 45 minutes. The product was cooled at room temperature for 1 hour and stored at 4 °C. For the next step, the result of the cooling process was dried in the oven at 50°C, milled, sifted, and analyzed its %RS.

In the hydrolysis by pullulanase, rice flour was dissolved in water (25% w/v). The mixture was autoclaved at 121°C for 20 minutes, then cooled to 60°C. The pullulanase enzyme was added into the paste, and then was heated at temperature 60°C by continuously stirring. After the certain hydrolysis time, it was heated to 100 °C, and cooled to room temperature. The product was then stored at 4°C, dried in an oven at a temperature of 50°C, milled, sifted and analyzed for its %RS.

3 Results and discussion

3.1 Analysis of viscosity and temperature during gelatinization of native rice

The measurement was done on 1% native rice flour (dry basis) using Rheometer instruments (type of spindle RV2) and rotation speed of 100 rpm. Observations of viscosity was made of temperature 30°C to 78°C with the rate of increasing temperature 2°C. The result of the measurement was shown in Fig. 1. Native rice that used in this research has a maximum viscosity of 12.6 m.Pa.s, at 66°C. Peak temperature is referred as the gelatinization temperature. According to Whistler R.L [7], gelatinization temperature of native rice starch ranges between 61-77°C, so the measurement results was in the range of data from literature.

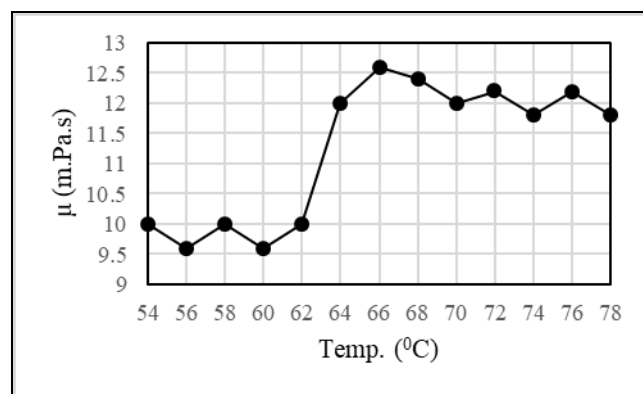


Fig. 1. Viscosity change during gelatinization of the native rice.

3.2 Analysis of resistant starch content (% RS)

3.2.1 Native rice

Based on the measurement in this study, resistant starch content in native rice used was 3.69%. This results showed that the native rice has a medium resistant starch

content, because it is in the range of 2.5 to 5% according to the grouping by Goni et al [8].

3.2.2 Acid hydrolysis process

The results of acid hydrolysis process with various types of acid and concentration, can be seen in **Fig. 2**. From the results obtained, it has not been seen any data tendency on the variation of acid type nor acid concentration used. The discrepancy of the results of this study may be due to the gelatinization process that was not done beforehand, so that the acid solution used was difficult to penetrate perfectly into the granules and cut off the branch bond. From this result it can be concluded that the gelatinization process has an important role on the modification of starch by partial hydrolysis process.

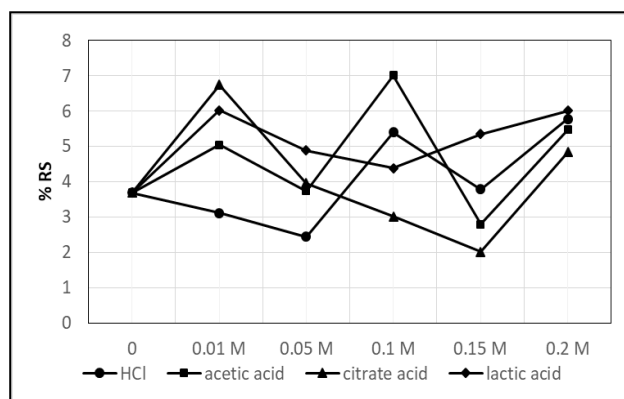


Fig. 2. %RS after the partial hydrolysis process at the various types and concentration of acid.

Resistant starch content as a result of acid hydrolysis process from this study was ranged from 2.01% - 7.02%. The %RS of some products increase against the %RS of native rice, but some products decrease. The results of hydrolysis using hydrochloric acid provide a significant improvement. It was predicted this acid was too strong in cutting off chains, so that, it produced monosaccharides that can dissolve in water.

3.3 Autoclaving cooling process

The resistant starch of modified flour obtained was in the range from 3.66 to 7.45%, shown in the **Fig. 3**. %RS of the some samples were doubled from native rice. But the result has not reached the same result as obtained by Edmonton and Saskatson [3]. They worked in Barley starch and done more than 1 cycle autoclaving and cooling process. By using only 1 cycle, the possibility of all amylose chain release from the granules is low. It will affect the amount of amylose-amylose and amylose-amylopectin that join back when retrogradation process are fewer so that the resistant starch content formed were also low [9].

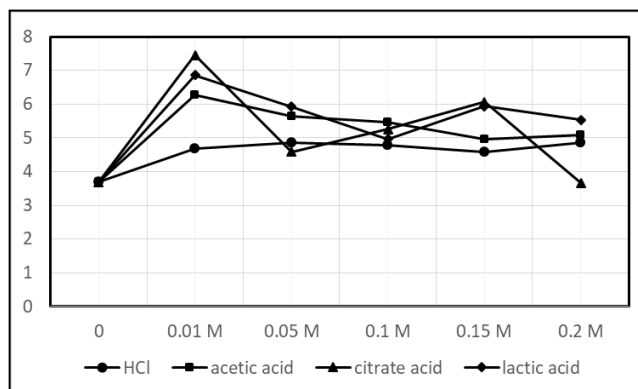


Fig. 3. %RS after the acid hydrolysis process followed by autoclaving & cooling.

From **Fig. 3** above, it can be seen that the highest yield of %RS, occur in starch modification with citric acid. According to Xie et al. [10], citric acid as anhydrous can react with the starch, producing esterified starch (chemically modified starch). Space barrier in ester group protects the glycosidic bond of amylose. Therefore, the resistance of amylose can be strengthened and the yield of %RS can be improved [11]. As for hydrochloric acid, it can be seen that the increased concentration did not give a significant influence on the increasing of %RS. It is in parallel with the result from the previous process. Since the hydrochloric acid is too strong, it can cut off the polysaccharide chains up to monosaccharides or weaken the glycosidic bonds. Therefore, in the autoclaving followed by cooling process, this chains will be cut off especially at the amylose molecules. As the result the resistance starch changed was not significant.

It can be seen also there was the tendency of resistant starch content (%RS) increased in acid hydrolysis followed by autoclaving cooling process. So, it means this process has a contribution to extend the increasing of %RS.

3.4 Pullulanase hydrolysis process

The content of resistant starch after pullulanase hydrolysis process from this experiments ranged from 6.43% - 7.61%. Effect of pullulanase hydrolysis time against the yield of resistant starch content, shown in **Fig. 4**.

From the results obtained, it can be seen that the resistant starch content (% RS) increased with the increasing time of pullulanase hydrolysis. This tendency is the same with the observation of Zhao and Lin [12]. The maximum yield of %RS was 7.61%, occurred at 8 hours of hydrolysis time.

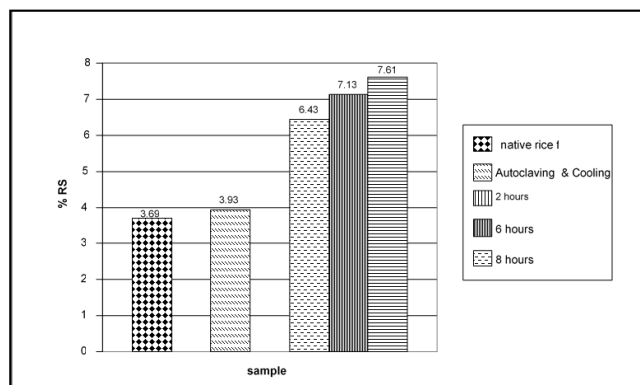


Fig. 4. %RS after the pullulanase hydrolysis with the variation of hydrolysis time

If the yield of resistant starch content from all processes was compared, it can be seen that pullulanase hydrolysis was more effective in increasing the levels of resistant starch content (%RS) compared to the acid hydrolysis and autoclaving-cooling process. All the resistant starch from pullulanase hydrolysis process in this study can be classified in the category of starch with high resistant starch content according to the grouping by Goni *et al.* [8] i.e. 5-15%.

4 Conclusion

The %RS in native rice is only slightly increased by acid hydrolysis process. The problem is difficult to get a homogeneous mixture during process since it is a liquid – solid phase.

Autoclaving-cooling process after acid hydrolysis process gives a contribution in increasing %RS after the acid hydrolysis process. But, if this process was done directly to the native rice the result is not too good.

Pullulanase hydrolysis process shows a good result in increasing of %RS of native rice. The increasing of %RS in parallel with the increasing hydrolysis time. From the variation of time selected in this research, the longest hydrolysis time i.e. 8 hours gives the highest resistant starch content i.e. 7.61%, obtained from pullulanase hydrolysis process with hydrolysis time for 8 hours.

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