

Sodium hydroxide (NaOH) concentration and steeping time duration effects on starch production from dry-milled low quality rice IR 64 grade 3 flour using alkaline-protease enzyme digestion method

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Abstract

The low quality rice, such as IR64 grade 3, is processed to produce the rice starch. The production process of the rice starch from low quality rice, IR64 grade 3, is investigated. The process is based on the alkaline protease digestion and alkaline steeping method. The main objective is to obtain high starch content and very low protein content. The enzyme in the process is produced by the fermentation activity of the *Bacillus megaterium*. The independent variables of this process are steeping time duration and Natrium Hydroxide (NaOH) concentration. Based on the protein content level and the amount of the produced waste, the combination between alkaline steeping and protease-digestion methods produces better quality product compared to the alkaline steeping method. High purity rice starch was produced at conditions 0.05 M NaOH solution and 7 hours steeping duration. The proximate composition of the produced starch has 87.92 % starch, 1.37 % protein, 0.42 % fat and 9.81 % moisture.

Keywords

Rice Starch
alkaline protease digestion
low quality rice
IR64 grade 3

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Introduction

One of the solutions to increase the economic value of low quality rice is to convert it into the rice starch. The rice starch is known as a food additive to give slower-digesting effect. Athletes like marathon runners build their endurance by eating the food containing the rice starch to keep a steady flow during the food digestion in the stomach. The rice starch is also added to the baby formula to thicken it for helping the baby with a fuller feel and a longer digestion process.

The rice starch is a unique starch among available commercial starches because of its small granule size and its hypoallergenic residual protein (Schoch, 1967). However, the rice starch at present time is less favorable than other starches due its higher production cost.

Starch is a mixture of two glucose polymers which are amylopectin and amylose. Amylopectin is a polymer having α -1,4-linked and α -1,6-branched (4-6% branching) structure with an average molecular weight near to 10^8 (Juliano, 2003). Amylopectin is formed by 100.000 glucose monomer. Amylose is composed by α -1,4-linked glucose units. The molecular weight of amylose is approximately 10^5 (Juliano, 2003). Amylose is formed from 500-20000

glucose monomer.

The molecular weight of starch polymers varies from plant to plant. Amylopectin molecular weight varies from 50×10^6 to 500×10^6 , and amylose ranges from 1600 to 106. Common starches typically contain 25-35% amylose and 65-75% amylopectin, The ratio between amylose to amylopectin ratio determines the characteristic of the starch. The more amylopectin in the starch, the stickier the starch will be. Rice starch contains 17% amylose and 83% amylopectin.

The shape and size of starch granules depend on the source and the ambient condition of the growing area. Starch shapes have the forms of globular, ellipse, oval, lenticular and amorph. The sizes of starch granules are about 3 to 30 μm for cereals about 10 to 100 μm for tubers.

When granule starch is mixed with water, the starch will gelatinize. In the gelatinizing process, the starch molecules swell irreversibly. Water can penetrate into a starch molecule depending on the temperature. The increase of the temperature leads to the increase of the kinetic energy to be greater than the bond energy within the starch molecules. At the temperature of 40 – 50°C, starch granules start absorbing the water to produce large starch granules. When the temperature reaches 50 – 60°C, the water content in the starch will be excessive and the water transfer occurs reversibly.

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When the temperature is raised until 80°C, the starch granules are gelatinized.

Starch can be hydrolyzed into smaller molecule using two methods. They are acid hydrolysis and enzyme hydrolysis. Enzyme hydrolysis is more common in industrial application because it is more profitable. Hydrolysis enzyme can be processed in a non-extreme condition (pH and temperature). Beside that, the liquid waste that is formed during the process is less than the other method. The process control is much easier to be executed and the rate of conversion is higher. Common enzymes that re used during the process are α -amylase, β -amylase, glucoamylase and pullonase. These enzymes are produced by bacteria, yeast, and vegetables. Every enzyme has its own characteristic during the starch hydrolysis process.

Starch promotes the physiochemical changes during the food process. This event is determined by the starch-water interaction, fat content, time, temperature, mechanical force, and biochemical activity. Starch has stable characteristics in liquid and other solvent. This characteristic is very suitable for preventing crystallization in the food product especially ice cream and others frozen products. Based on this characteristic, starch can form a good texture in many food products.

Rice usually contains 80-90% starch. It becomes one of many potential sources of starch. Unfortunately, the information about the production process of rice starch from low-quality rice is limited. This research is then motivated by this reason especially to utilize IR64 low grade rice as the raw material to produce the rice starch. The ultimate goal of this research to contribute on establishing the rice-starch process technology.

There are several methods for producing rice-starch. They are alkaline-steeping method, high-intensity ultrasound and surfactant, Guraya method, protease digestion and alkaline-protease methods,. The alkaline steeping-method (Yang *et al.*, 1984) is the simplest method for starch production. This method is applied easily in a simple apparatus system. The advantage of this method is high purity of produced starch. The disadvantages are that the effluent contains high concentration of salt and higher percentage of damaged starch (Schoch, 1967). This leads to higher cost on the waste treatment.

The detail mechanism of rice protein extraction into alkaline solution has not yet been clearly defined. The mechanism is probably based on the protein solubilization in the alkaline solution without chemical changes. The extraction process continues and stops when the equilibrium is reached. This assumption is based on the protein extraction process

in the animal tissue (Alpert and Schimerr, 2000) The soluble protein can be isolated effectively.

High-intensity ultrasound and surfactant methods (Wang and Wang, 2004) degrade the starch into narrow molecular weight. This starch is a native starch and partially hydrolyzed starch in aqueous dispersion. The dispersion or suspension of the starch in the solution is subjected by the action of ultrasound in which it is degraded by the sonic doses.

Guraya method was developed by (Guraya and James, 2002). Guraya's approach relies on very high pressure that is supplied by a special homogenizer known as a micro-fluidizer to physically split apart the starch-protein agglomeration. A single pass through a piece of equipment yields small and individual particles of starch and protein. Starch and protein are homogeneously dispersed in a watery matrix. The starch and protein components can then be separated by a traditional density-based separation processes.

Proteases are the enzymes which play the role of biocatalysts in the organism. They catalyze the reactions of all metabolic processes. Proteases are involved in splitting the peptide bonds which link the amino acid. These enzymes digest long protein chain to shorter fragments. The process begins with the hydrolysis of the peptide bonds that link amino acids together in the polypeptide chain into smaller molecules.

Protease digestion method employs an enzyme to digest the protein in the rice flour (Juliano, 1991; Zheng and Bhatt, 1998). This process usually undergoes at neutral pH and requires longer time duration. For this reason, the protease digestion method was improved by (Lumdubwong and Seib, 2000) by increasing the pH of the protease to digest the rice endosperm. Higher pH will enhance the hydration of the protein. This improved method was called by the authors as the alkaline protease digestion method.

Another variation of the method was investigated by (Puchongkavarin *et al.*, 2005). Their study employed a serial enzymatic process using cellulase at slightly acid condition and protease under neutral condition to produce the rice starch. The used rice was long-grain polished rice. They produced slightly higher protein content with less starch damage.

This paper reports the results of our research on the investigation of the alkaline protease-digestion method adopted from (Lumdubwong and Seib, 2000) for starch production from low quality rice of Indonesia Rice IR 64 grade 3. The rice IR 64 grade 3 was directly obtained from the market and milled in the dry condition without any treatment. Lumdubwong and Seib milled the rice in the wet condition. The alkaline protease-digestion method

works on the basis of protein extraction through the mechanism of the digestion reaction by the solvent and protease enzyme activity. The produced starch has the quality as good as the available commercial rice starch.

Alkaline-protease digestion method in this research is a modification method of alkaline protease. Protein extraction process that occurs in alkaline protease digestion is optimized using the protease enzyme. Protease enzyme is used to decompose the protein content in the rice starch. This enzyme is also useful to reduce the toxicity of alkaline waste that is produced during the process.

Materials and Methods

The rice starch is usually produced through either alkaline solution steeping method, protease enzyme digestion method or combined alkaline and protease enzyme digestion method. Basically, these methods extract or digest the protein contained in the rice. The whole process involves (i) mixing of rice flour with alkaline solution or with *Bacillus megaterium* bacteria or both alkaline and bacteria, (ii) digesting of the protein, (iii) separation with centrifuge between solid and solution, (iv) washing with water, (v) neutralizing pH with HCl acid solution and (vi) drying the starch product.

The enzyme used in this research was produced by fermentation technology from *Bacillus megaterium* bacteria. *Bacillus megaterium* are rod shape bacteria. They are considered as large eubacteria found in the soil. Groups of the bacteria are often found in the chains where the cells are joined together by polysaccharides on the cell walls. *Bacillus megaterium* are able to survive in some extreme conditions such as desert environments. The enzyme has the catalytic activity at 10,000 unit/mm, the active pH at 8 to 10 and active temperature at 25 to 37°C.

Instead of using bacteria alone, the alkaline solution is also added to digest the rice flour. This process refers as an alkaline protease method. This experiment focused on the alkaline protease method. This method was adopted from (Lumdubwong and Seib, 2000) with some modification. The experiment involve both production and product analysis stages.

The materials and chemicals for the production process are dry-milled rice IR64 (low grade Indonesia Rice), sodium hydroxide, and protease enzyme. The protease enzyme was obtained from Puspitok Serpong. Others are Nelson reagents, hydrochloric acid 25%, Kjeldahl salt, sulphuric acid, and boric acid. The commercial rice-starch was obtained from

Cho-Heng Vermicelli Rice Factory Co Ltd Thailand.

The production apparatus are milling machine, magnetic stirrer, centrifuge, pH meter, and oven. A milling machine was used to grind the grain rice into the rice flour. Magnetic stirrer blends rice flour and sodium-hydroxide solution to form a slurry. Meanwhile a centrifuge was used to separate the solid from the liquid. The level of pH was measured by a pH meter. The starch product was dried in the oven.

The instruments for the product analysis include centrifuge, reflux unit, electric heater, pH meter, spectrophotometer UV, Kjeldahl extractor, distillation unit, titration unit, and vacuum oven. A set of centrifuge system (reflux unit, electric heater, pH meter, and spectrophotometer UV) was applied to measure the starch content. The Kjeldahl extractor, distillation unit, and titration unit were dedicated to determine the protein content in the produced rice starch. A vacuum oven was used to determine the moisture content from the produced starch.

The starch production process starts with grinding low-quality rice IR64 using a dry miller. The rice flour, 40 grams, was mixed with 100 ml NaOH in various NaOH concentrations and stirred to form a slurry. The protease-enzyme was added to the slurry. The temperature was maintained between 25-37°C. The starch-NaOH mixture is then centrifuged at 4000 rpm for 15 min and the dark yellow supernatant was discarded. The sediment was then washed with 100 ml demineralized water, centrifuged at 4000 rpm for 15 min. Then the supernatant was removed. The solid part is mixed with another 50 ml of demineralized water by adjusting its pH to 7 with HCl solution. The neutralized solution was centrifuged at 4000 rpm for 15 minutes and the supernatant was removed. The dark tailings layer at the surface of the starch was scraped manually using a metal spoon. The produced starch was finally washed using 100 mL demineralized water, centrifuged at 4000 rpm for 15 minutes and the supernatant was removed. The tailing was discarded, and then the starch dried up on the oven for about 48 hours. The production flow process is constructed in Figure 1.

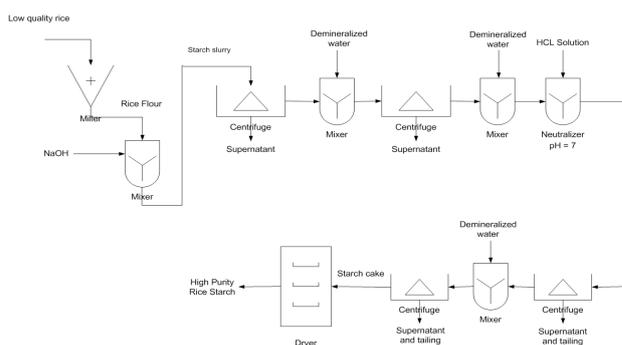


Figure 1. Flow chart of the rice starch production process

In order to determine the conditions for the rice-starch production, NaOH concentration and steeping time duration were varied. The concentration of sodium hydroxide was varied 0.1, 0.2, and 0.4% mass or equivalent to 0.025M, 0.05M, 0.1M concentrations. The steeping time variables were 3, 5 and 7 varied hours.

The NaOH concentration variations are limited between 0.1% w/w and 0.4% w/w. Our previous experiments shows that the optimum condition for alkaline-steeping method was 0.4% mass of NaOH. Gelatinizing occurs at high concentration of NaOH. This situation obstructs the extraction process (Yamamoto, 2004). There were 9 runs executed in this experiment. Each concentration of sodium hydroxide was run with 3, 5, and 7 hour time duration.

Produced starch was analyzed for its proximate content. This includes the measurement of the starch, protein, moisture, fat, and ash contents. The fat content was measured using a soxhlet extractor.

Results and Discussion

Effects of NaOH concentration and steeping time duration

The effect of NaOH concentration to starch content at various steeping time duration is shown by Figure 2. The figure shows that the optimum performance of alkaline protease enzyme is reached at 0.05M NaOH concentration and 7 hours steeping duration to produce highest starch content.

The effect of steeping duration to starch content at various NaOH concentration is presented by Figure 3. The best condition is obtained at 7 hours for 0.05M NaOH concentration. This means that this enzyme work effectively to digest the protein contained in the low quality rice.

The quality of produced starch is indicated by higher starch content and lower protein content. The best quality of the produced starch is obtained for the Sample 13. The sample contains starch above 85%. The proximate analysis results of the produced starch is given in Table 1.

Table 1. Comparison produced starch (Sample 13) to commercial rice starch and IR 64 grade 3 flour

Component	Commercial Rice Starch	Sample 13	IR 64 flour
Moisture (%)	9.5	9.81	11.16
Starch (%)	88.68	87.92	72.33
Protein (%)	1.15	1.37	8.71
Fat (%)	0.19	0.42	0.42
Other components	0.48	0.48	7.38

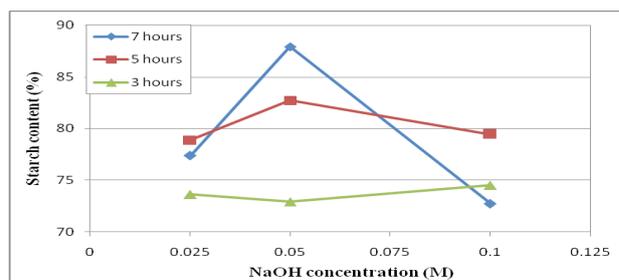


Figure 2. Correlation between starch content and NaOH concentrations in alkaline protease-digestion method

It is interesting to discuss about the interaction effect between NaOH concentration and the steeping time duration for the alkaline-protease method. For alkaline method only, the starch content increases with NaOH concentration. However, for alkaline-protease method, there is an optimum NaOH concentration to produce a maximum starch content at each certain steep time duration (Figure 3).

The longer the steeping time duration, the higher the starch content that is produced. This applies to the NaOH concentration at 0.05M. Without the protease enzyme, the starch content is always higher for higher steeping time duration. It seems to be that the protease enzyme effectively works at certain NaOH concentration. NaOH concentrations at 0.1M and 0.025M, the protease enzyme work poorly after 5 hour digestion process (Figure 3).

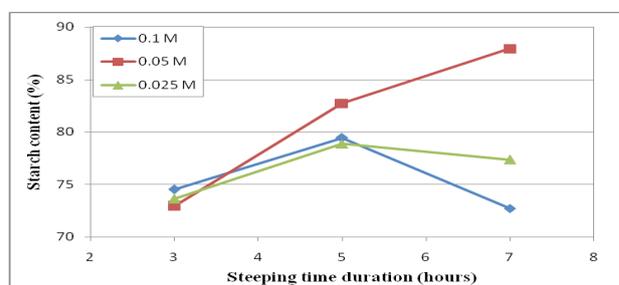


Figure 3. Correlation between starch content and steeping time duration in alkaline protease-digestion method

Protein content

Since the Sample 13 has less protein content at the value of 1.37%. This sample was used for a comparison analysis in finding the correlation between protein content with NaOH concentration and steeping time duration.

The protein content is the second response variable to determine the quality of the rice starch. The protein content must be kept as low as possible to meet the specification of high quality rice starch. High content of protein could spoil the sticky and gristly characteristic of the rice starch.

In order to construct the correlation between

protein content and NaOH concentration, Samples 10, 13, and 16 were analyzed. These samples were produced with the same method and the same steeping time duration. The result for the correlation between protein content and NaOH concentration shows that the increase in NaOH concentration leads to the decrease in the protein content.

Samples 13, 14, and 15 were analyzed for presenting the correlation between the protein content and steeping time duration. Our results show that the protein content decreases with the steeping time duration. The lowest protein content was resulted at 7 hour steeping duration.

Gelatinizing analysis

Two parameters that are usually used for gelatinizing process. These are viscosity and gel strengthness. In this work, the gelatinizing analysis is based on a visual observation due to the difficulty in measuring the gelatinizing viscosity.

When the gelatinizing process occurs, the starch forms a gel condition due to high viscosity. A good quality of starch is identified by a homogeneous gel. When the gelatinizing condition is achieved, there is no appearance on any tiny granule. For IR 64 rice flour, the color is white with some yellow shadow and tiny grandules were found in some part of the gel. For produced starch Sample 13, the color appearance is dominantly white and transparent.

Based on the visual observation, the low quality rice and Sample 13 have slightly dark yellow color. This phenomenon is caused by the existence of small particel of soil, dirt and other impurities that were unsuccessfully removed and washed during steeping process.

Optimum condition

Optimum condition is determined by the variables of NaOH concentration and steeping time duration. The optimization objective is to have the starch content as high as possible and to keep the protein content as low as possible. The protease digestion shows that the optimum condition is at 0.05 M NaOH concentration. It is an indicator for the protease enzyme that works effectively in this condition. This result also proves that the enzyme succesfully reduce the consumption of NaOH. The usage of protease enzyme in this work has a considerable effect in reducing the protein content.

According to the data obtained above, the optimum condition to produce a high quality rice starch from the raw material of low quality rice with the alkaline protease digestion method is resulted at 0.05M NaOH concentration and 7 hours steeping time duration. Sample 13 has 89.72% starch content

1.37% protein content. Commercial starch is slightly brighter than the Sample 13. Both of them have a similar texture.

Comparison

There are several research results reported in the literatures about the rice starch production for different methods. Each of them gives different results. These differences are caused by some factors, such as rice flour content, rice quality, pre-treatment, production method, separation method and others.

Table 2 shows the comparison between the present work results with the other similar studies. The results of (Wang and Wang, 2001) were achieved at low NaOH concentration, 0.1% mass NaOH, but longer steeping time duration, 18 hours. The present results were obtained at 0.2% mass NaOH and 7 hours steeping time duration. The longest steeping duration was employed by (Wansuksri, 1999). The results that are reported by (BIOTEC Thailand, 2012) were achieved at higher NaOH concentration than the present results.

The produced starch in this work contains 1.37% of protein. This result is slightly higher by comparing to others. This phenomenon may be caused by the differences of material and with no pretreatment. The present work uses a low quality rice that contains higher protein and impurities while the other researches used a selected and pre-treated rice. High quality rice's color is bright white. This is a sign that it contains less protein than the low quality rice.

Table 2. Rice starch comparisons

No.	Work	NaOH optimum concentration	Steeping Time Duration (Hours)	Stirring Process	Protein Content (%)
1	Present using Alkaline-Protease Digestion method	0.2% mass	7	yes	1.37
2	(Wang and Wang, 2001)	0.1% mass	18	No	0.1
3	(Wansuksri et al., 1999)	0.3% mass	24	No	86% of protein is reduced
4	(BIOTEC Thailand, 2012)	0.25-0.5% mass	5	Yes	<1

Conclusions

It is concluded that alkaline protease-digestion method was successfully implemented to produce the a better quality rice starch from low quality rice IR 64 grade 3 by using the ordinary bacteria for the enzyme production by the name of *Bacillus megaterium* from Puspitek Serpong. High purity rice starch was obtained using 0.05M NaOH solution within 7 hours of steeping duration. The result also shows that optimized protease enzyme succesfully reduces NaOH usage up to 50% weight. It leads to

the production waste to be less hazardous for the environment. Further improvement are needed to produce high quality rice starch that meets the international starch standard. The future research should emphasize on using different enzymes that can be active in the neutral condition.

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