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Degree of Hydrolysis of Proteinaceous Components of Porang Flour Using Papain

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***Abstract*—**Glucomannan can be found in the tuber of porang together with starch and proteinaceous components which were regarded as impurities. An enzymatic process for obtaining higher glucomannan content from Porang flour have been conducted. Papain was used for hydrolysing proteinaceous components in Porang flour which was conducted after a simultaneous extraction of glucomannan and enzymatic starch hydrolysis. Three variables affecting the rate were studied, i.e. temperature, the amount of enzyme and the stirring speed. The ninhydrin method was used to determine degree of protein hydrolysis. Results showed that the rising of degree of hydrolysis were fast in the first ten minutes of the reaction and then proceeded slowly afterward. The optimum temperature for hydrolysis was 60 ^{o}C. Increasing the amount of enzyme showed a remarkable effect to degree of hydrolysis, but the stirring speed had no significant effect. This indicated that the reaction controlled the rate of hydrolysis.

***Keywords*—**Degree of hydrolysis, ninhydrin, papain, porang flour, proteinaceous components.

I. Introduction

GLUCOMANNAN is a natural abundant polymer which can be extracted from the corm of *Amorphophallus sp*. *A konjac* is grown in China and Japan but in Indonesia, porang (*A. muellery* Blume) is used as source of glucomannan. Glucomannan is composed of β-1,4 linked D-mannose and D-glucose with ratio of the monomers is 1.6: 1. Acetyl group attached at the C-6 of the sugar residue approximately at every 9 to 19 residues [1]. With its very high molecular weight (200000 – 2000000), glucomannan possesses highest viscosity at 1% solution among the other natural polymers [2].

Even nowadays glucomannan is mostly used as healthy food for its zero calorie, its utilization will be broader in the future as glucomannan is a promising polysaccharide. Many researchers were interested in utilizing it as a pharmaceutical excipient, for example, as material for oral colon targeting drug delivery system (OCDDS) [3]. For this purpose, a pharmaceutical grade of glucomannan with 100% purity is needed.

Polysaccharides present in its sources with the other substances. Depending on cultivars, the konjac corm dry matter mainly composed of glucomannan (around 60%), starch (around 20%), protein (around 10%), lipid and minerals [4]. Glucomannan is contained in a bag which is surrounded by smaller starch granules. Starch in konjac tuber is considered as impurity since its presence can reduce viscosity and increase turbidity [5]. In the production of konjac flour, the washed konjac corms are sliced into thin chips, and the chips are dried in the hot-air drier. The dried chips are ground and then, the glucomannan particles (konjac flour) are obtained. The glucomannan particles are bigger and heavier than starch particles and they can be separated by wind shifting or shieving. [6]. The produced flour contains glucomannan up to 70% (w/w). Purified or extracted glucomannan contains more than 90% glucomannan [8]. Several techniques used in the extraction process include washing with ethanol, using enzyme, dialysis, coagulation using aluminum sulphate, and their combination [7]. Fadilah [9] used amylase to hydrolyze starch in porang flour which was done simultaneously with extraction of glucomannan.

Removal of protein to obtain purer polysaccharides was done by Sevag method for purification of glucomannan [9], gellan gum [10], mannan oligosaccharides [11], and Chinese yam polysaccharide [12]. Sevag method seems to give good result, but the reagent contains poisonous chloroform that is harmful to environment. This may cause a bad impact especially if the polysaccharides used as an additive in food.

To handle the weakness of chemical treatment, enzymatic process for deproteinization is necessary as it is environmentally friendly in replacing the harsh chemical method in purification of polysaccharides. Wang [10] successfully used alkaline protease in deproteinization of gellan gum and found that the protein removal was 89.3% where the residual protein concentration of gellan gum was 0.125% (w/w).

Enzymatic process for deproteinization in order to have purer glucomannan was done by Khanna [13] which uses Promod 278 P. Unfortunately, there is no report on how much protein could be removed in this study. In the design of process equipment, it is necessary to have the information about how much and how fast protein can be hydrolyzed by an enzyme. For this reason, it needs to evaluate the extent of reaction which can be done by measuring the degree of hydrolysis (DH).

In order to isolate and to obtain purer glucomannan, a series of processes which involves enzymatic hydrolysis of starch followed by enzymatic hydrolysis of proteinaceous components of porang flour has been conducted. The action of papain on the proteinaceous component of porang flour was investigated. The influence of three variables namely

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temperature, amount of enzyme and stirring speed on the degree of hydrolysis were studied.

II. Materials and Methods

A. Material

Porang corms were harvested in Madiun, East Java, Indonesia. The corms were washed, peeled and sliced into 5 mm thickness. The sliced corms were then immersed in 1% sodium bisulphite solution for 1 minute before dried in a cabinet dryer at 60 ^{o}C for 24 hours. The dried corms were then ground and sieved. The powder which pass 425 µm sieve aperture was collected as porang flour. α-Amylase (*Bachillus licheniformis*, CAS 900-85-5) was obtained from Sigma-Aldrich. Papain (*Carica papaya*, EC Number 3.4.22.2) was obtained from Sigma-Aldrich. Buffer phosphate solution at pH 6 used as carrying process medium.

B. Hydrolysis Process

The hydrolysis of proteinaceous components was conducted after the simultaneous extraction of glucomannan and hydrolysis of starch. Twenty gram of porang flour was added into a 2000 mL of 50% ethanol solution and magnetically stirred. The washing step was conducted for 90 minutes. The solid was then removed from the solution. This resultant solid was then added into 2000 mL of 70 ^{o}C buffer phosphate solution which contained a certain amount of amylase. The porang flour underwent a simultaneous of extraction and starch hydrolysis process. This process was conducted with constant stirring speed at 500 rpm for 90 minutes. When this process had finished, the solution was set into a condition for hydrolysis of proteinaceous components. An amount of papain was dissolved in the buffer solution and then added into the solution. At a certain time interval, 10 mL of sample was taken out and heated in a boiled water for 10 minutes. The sample was then centrifuged to remove the undissolved solid. The supernatant was then analysed for the amino acid content by using ninhydrin method. For studying the effect of temperature, the conditions were set 50 ^{o}C, 60 ^{o}C, 70 ^{o}C, and 80 ^{o}C by keeping the stirring speed at 500 rpm, and the amount of papain added was 0.1 g. For studying the stirring sped, the conditions were set at 200 rpm, 400 rpm and 500 rpm while the temperature was set at 70 ^{o}C, and the amount of papain was 0.1 g. For studying the effect of amount of enzyme, the added papain was 0.1 g, 0.2 g, 0.3 g and 0.5 g with constant temperature at 70 ^{o}C and stirring speed at 500 rpm.

C. Evaluation of the Degree of Hydrolysis

The method of ninhydrin for determination of amino acid included coloring samples by adding ninhydrin reagent and heating it up at 100 ^{o}C, from which a blue-purple color developed. The intensity of the color was then measured at 550 nm. The amount of amino acid released (h) was then calculated by subtracting the value of unhydrolysed sample. The degree of hydrolysis, DH, was determined from:

$$DH = \frac{h}{h_{tot}} \times 100\% \quad (1)$$

where h_{tot} is the total amount of peptide bonds. The value of h_{tot} for substrate that remains unknown can be estimated by 8 amino meq/g protein [14].

III. Results and Discussion

Initially the formol titration method was chosen to determine the amount of amino acids released during the hydrolysis. Nevertheless, the amount of amino acids released was too small to detect by formol titration and made it hard to identify the equivalence point. Furthermore, the results of the formol titration gave the value of DH more than 100%. With these results, the determination of amino acids was then done by using the ninhydrin method. This method showed better results for determining less amino acids content.

Figs. 1-3 show the changes in the DH value as the reaction took place. As can be seen, the DH value increases rapidly in the beginning of the reaction then tends to be constant. The rate of hydrolysis (dh/dt) decreases with time. According to [15], the decrease in the rate of hydrolysis probably was due to: (1) the decrease in the concentration of peptide bonds susceptible to hydrolysis; (2) substrates or products inhibition; and (3) enzyme inactivation.

Fig. 1 shows the effect of temperature on the hydrolysis of proteinaceous components of porang flour by papain. As it is seen, the temperature gave remarkable effect to DH. The rapid changing in DH for various temperature occurred in the first ten minutes of the reaction. The increasing of DH value from 0 to 1.26%, from 0 to 1.85%, from 0 to 1.18%, and from 0 to 0.63% were obtained for rection at temperature 50 ^{o}C, 60 ^{o}C, 70 ^{o}C and 80 ^{o}C, respectively. The highest DH values obtained were 1.59%, 2.34%, 1.69%, and 0.78% for the reaction at temperature 50 ^{o}C, 60 ^{o}C, 70 ^{o}C and 80 ^{o}C, respectively.

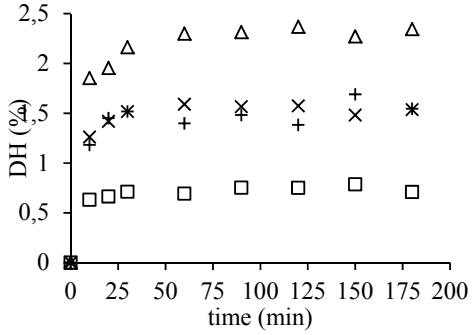

Fig. 1 Degree of hydrolysis of proteinaceous component of porang flour for various temperature (ϒ 80 ^{o}C , + 70 ^{o}C, Δ 60 ^{o}C, × 50 ^{o}C)

It can be seen that raising the temperature from 50 ^{o}C to 60 ^{o}C resulted in increasing DH, but raising the temperature from 60 ^{o}C to 70 ^{o}C and 80 ^{o}C resulted in decreasing DH. This is probably due to the inactivation of enzyme as the temperature rose. It can be said that the optimum temperature of the reaction was at 60 ^{o}C since DH achieved at this temperature has the highest values. This result is different from what

Damrongsakkul [16] obtained (the activity of the papain at 70 ^{o}C was higher than at 60 ^{o}C).

The influence of the amount of enzyme on the DH was investigated by using amount of enzyme in the range of 0.1 g to 0.5 g, and the results were given in Fig. 2. Again, the rapid changing in DH obtained in the first ten minutes of the reaction. For 0.1 g, 0.2 g, 0.3 g, and 0.5 g papain added, the increasing DH values were from 0 to 1.18%, from 0 to 1.87%, from 0 to 5.2%, and from 0 to 7.63%, respectively. The DH value increased as the amount of enzyme increased. The highest DH values obtained were 1.69%, 3.03%, 5.66%, and 8.5%. for the adding of 0.1 g, 0.2 g, 0.3 g, and 0.5 g papain, respectively.

For maximum DH obtained, increasing amount of enzyme from 0.1 g to 0.2 g resulted in increasing DH by 1.79 fold, from 0.1 g to 0.3 g resulted in increasing DH by 3.35 fold, and from 0.1 g to 0.5 g resulted in increasing DH by 5.03 fold.

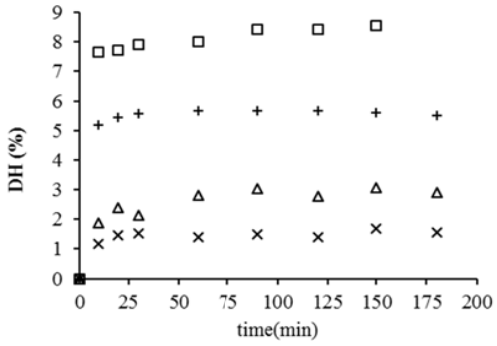

Fig. 2 Degree of hydrolysis of proteinaceous component of porang flour for various amount of enzyme (□ 0.5 g, + 0.3 g, Δ 0.2 g, × 0.1g)

The influence of stirring speed on the hydrolysis of proteinaceous component in porang flour by papain is presented in Fig. 3. As can be seen, the DH values obtained at various speed of stirring speed were found slightly different. For stirring speed at 200 rpm, 400 rpm and 500 rpm, the DH values in the first ten minutes increased from 0 to 1.13%, from 0 to 1.32% and from 0 to 1.82%, respectively. The highest DH values were 1.37%, 1.51% and 1.69% for the stirring speed 200 rpm, 400 rpm and 500 rpm, respectively. This slight difference in DH values for all various speed of agitation showed that the overall process of hydrolysis was controlled by the reaction. Although not the controlling factor in the rate of hydrolysis, agitation was needed to enhance the contact between enzyme and substrate.

For the all experiments, the highest DH was only 8.5% which was obtained at 70 ^{o}C, stirring speed at 500 rpm, the amount of papain was 0.5 g, and after 180 min of hydrolysis. It is difficult to compare the value of DH in this study with those from the data in the literature since no authors used the same substrate and enzyme in their experiments. However, some authors reported the DH value using papain for the hydrolysis of sea urchin gonad which was 18.35% for 180 min of hydrolysis [17], palm kernel cake protein which was 87% after 30 h of hydrolysis [18], and sweet potato protein was 4% after 4 h of hydrolysis [19].

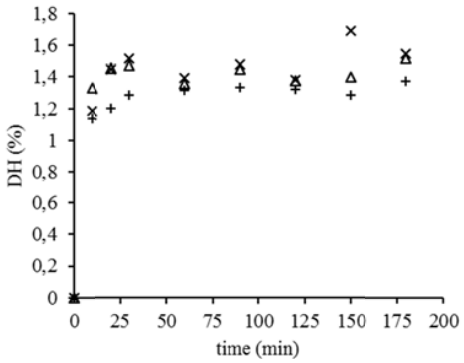

Fig. 3 Degree of hydrolysis of proteinaceous component of porang flour for various stirring speed (× 500 rpm, Δ 400 rpm, + 200 rpm)

IV. Conclusion

Proteinaceous components of porang flour can be hydrolysed by using papain. The ninhydrin method used to determine the degree of hydrolysis. In order to study the influence of operation condition, the hydrolysis of proteinaceous components of porang flour by papain was performed at various temperatures, amount of enzymes, and stirring speeds. The optimum temperature was 60 ^{o}C. The degree of hydrolysis changed proportionally with the amount of enzyme but the stirring speed had no significant effect.

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