RESEARCH ARTICLE

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Protein Degradation and Structural Change of Calcium Oxalate in Wet Conjuct Flour, Dry Conjuct Flour and Conjuct Soap

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Amorphopallus muelleri or Conjuc flour is generally use in cosmetic industry for skin smoother. However it is also contain high number of calsium oxalate crystal, which trigger skin itchyness.The aim of this study is to investigate conjuc powder capability in degrading protein and changing its calcium oxalate crystal structure in dry and wet of conjucpowder, during its process into cosmetic soap. The collected conjuc tuber was brought into laboratory to be milled into powder. Some of this powder was dried in oven to reach its steady dry weight. The other some of dry powder then processed into cosmetic soap. Capability in protein degradations were analyze by calculating the persentage decrease of treated albumin. Its capability in changing calsium oxalate crystal structure was monitored under microscope. The study was conducted using a completely randomized design (CRD) with a single factor, and each treatments were repeated three times. Resulted data was analyzed by ANOVA with a significance level of 95%. Results showed that during processed into cosmetic soap, there was no significant different in protein degradation, but significantly affect on number and structural change of calcium oxalate crystal. It is suggested that, in process to be cosmetic soap, the itchyness effect can be alleviated.

Keywords: *Amorphopallus*, Protein Degradation, Calcium Oxalate, Cosmetic Soap.

1. INTRODUCTION

Porang (*Amorphopallus muelleri*) is a kind of tubers plant group that grow wildly in several places in Indonesia. Porang tuber has great potential, but not fully utilized properly. Whereas porang tuber contains high number of protease and glucomannan that are good for skin smoother, so that it has great potential in cosmetics industry.[1]

Porang (*Amorphophallus muelleri*) contains the highest level of glucomannan among other *Amorphophallus* species that is found in Indonesia. Porang has great potential and prospects to be developed in industry because the high glukomannan compound has high economic value.[2] Glucomannan can be used in various industries, such as paper, textiles, paint, negatives film materials, insulation materials, celluloid and cosmetics ingredients.[3] Fresh porang contains 3.58% glucomannan.[4]

Based on the results of research conducted by the Center Hall for Postharvest (2009) showed that fresh porang tuber contains water (79.62%), ash (0.65%), protein (1.49%), fat (0.35%), glucomannan (13.06%) and calcium oxalate (0.23%). From these data, it can be known that besides containing glucomannan, porang tuber also contain high protein. Protein and protease are interrelated; protease is an enzyme which its function is to break down protein into smaller structures, so that protease has properties such as protein. In Japan, porang has been used as a cosmetic product because it contains high protease which is used to rejuvenate skin.[5] However, the oxalate content in porang can trigger itching, so good porang is porang which contains low level of calcium oxalate. Calcium oxalate in porang tuber has needle-shaped, so that's tapered and flocking which is called as rafida.[6]

Based on those reasons, the study aims to characterize protein degradation and breakdown of calcium oxalate in wet conjuc flour, dry conjuc flour and conjuc soap. Protein degradation test is done to measure protein degradation ability of porang products and calcium oxalate observation is used to observe structural change of calcium oxalate in the wet conjuc flour, dry conjuc flour and conjuc soap. From those data, it can be seen whether the conjuc flour that has great potential to be developed in industry.

2. EXPERIMENTAL DETAILS

In this study, there are 2 observations, protein degradation test and structural change observation of calcium oxalate. The treatments are giving wet conjuc flour, dry conjuc flour, and cojuc soap that has been made to the tests. Protein degradation capability measurement is done in several stages, including; (1) 100 grams of wet conjuc flour is added with 100 ml of cold water. (2) Wet conjuc flour and water are homogenized in a blender, then filter it uses a filter paper to obtain clear conjuc

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water. (3) Separate white egg with egg yolk, white egg is taken. (4) Two treatments which are given: treatment 1 (5 ml of white eggs + 2 ml of water from wet conjuc flour) and treatment 2 (5 ml of white eggs + 2 ml of distilled water) as a control. (5) Each treatment with 3 repetitions. (6) All samples are boiled until boiling; the liquid portion of the sample is removed and spare parts of solid or viscous. (7) All samples are boiled, silenced about 10–15 minutes, and then weighing is done. (8) Decreased level of the protein substrate is measured by subtracting the weight of the control sample with treated sample of water from wet conjuc flour. (9) The same experiment is conducted on dry conjuc flour and conjuc soap with 3 repetitions.

Steps in structural change observation of calcium oxalates use micrographs including; making preparations and preparations observation. The steps in the making of preparations are:
(1) Glass object that has been cleared from fat is dropped of water in the middle of object glass.
(2) Wet conjuc flour is placed in the middle of the object glass that has been poured water, and leveled as thin as possible.
(3) Mixture is covered with a cover glass.
(4) Edge of a cover glass is given with masking tape.
(5) The solution that strays beyond the limits of the cover glass is cleaned.
(6) Excess water is sucked by tissue.
(7) The same experiment is conducted on dry conjuc flour and conjuc soap.

Steps in preparation observations including:
(1) Preparation is observed using micrographs with a magnification of 400×.
(2) Sampling is done with three repetitions using three different preparations.
(3) Each slide of preparation is observed with 4 times calculation of the preparations in the slide to the right, left, top, and bottom.
(4) Amount of oxalate needles and length of oxalate needle are calculated in each shift.

3. RESULTS AND DISCUSSION

3.1. Protein Degradation in Wet Conjuct Flour, Dry Conjuct Flour and Conjuct Soap

Based on the results of protein degradation ability in wet conjuc flour, dry conjuc flour, and conjuc soap show that conjuc soap has maximum value of protein degradation ability and wet conjuc flour has minimum value of protein degradation ability. However, ANOVA test show that differences in ingredients (wet conjuc flour, dry conjuc flour and conjuc soap) have no significant effect in improving the capabilities of protein degradation. Average of protein degradation ability in each treatment is presented in Figure 1.

Conjuc soap has the highest ability of protein degradation because there are so many steps in the process of making conjuc soap that accelerate protein degradation process such as boiling. Besides porang as the basic ingredients of the soap, it also contains a mixture of several ingredients that are a source of protein such as coconut oil, olive oil, and soybean oil. In addition, protease enzyme has a same structure as protein structure. The additional materials in the manufacturing of conjuc soap also contain protein; it can increase the content of protease enzyme in conjuc soap. Enzyme is a structural compound of protein that has a function as a catalyst and it's known as bio-catalyst. In addition, the

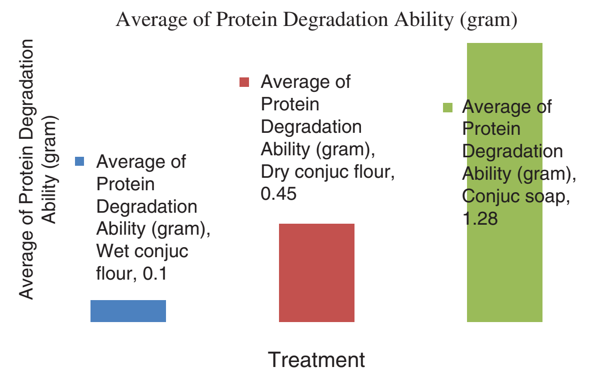

Fig. 1. Histogram average of protein degradation ability on wet conjuc flour, dry conjuc flour, and conjuc soap.

boiling process in the manufacturing of conjuc soap can accelerate the level of protein degradation.[7] Protein degradation is a process of protein breakdown from its bond. Protein degradation may occur due to heating or chemical contamination. It indicates that conjuc soap has potential to be developed in the cosmetics industry.[8]

3.2. Structure of Calcium Oxalate in Wet Conjuc Flour, Dry Conjuc Flour, and Conjuc Soap

3.2.1. Structural Change of Calcium Oxalate in Wet Conjuc Flour, Dry Conjuc Flour and Conjuc Soap

Based on observation data about structural change of calcium oxalate in wet conjuc flour, dry conjuc flour, and conjuc soap, it shows that there are differences fineness in oxalate needles are found. Oxalate needles that are found in wet conjuc flour has pointed needles and long size of calcium oxalate needles. While dry conjuc flour has blunt needles and shorter size of calcium oxalate needles and conjuc soap has no oxalate needles. Pointed or dulling of oxalate needles greatly effect on porang tubers itchy. The finer oxalate needle tip has itchier. Shape of calcium oxalate tubers are in the form of needle cristals of calcium oxalate. Needle prick of calcium oxalate causes itching in the mouth when we are eating tubers or we are peeling tubers skin.[9] Picture of calcium oxalate structure can be seen in Figure 2.

Based on Figure 2, it shows that calcium oxalate shape in porang is needle shaped. Calcium oxalate needles lead to trigger itchy. The itchy of tubers when we consume it because calcium oxalate needles that are packaged in transparent capsules contain different liquids between each cells of tuber. This capsule is called as rafid. Rafid are embedded in separation wall between two vacuoles on tuber tissue tips. The peeling of tuber epidermis, it leads to the rupture of capsule wall that is caused by the difference of voltage between each vacuole that contains liquid. As the effect, calcium oxalate appears and stabs the skin. This puncture which leads to the itching in the mouth, throat, and skin.[10]

3.2.2. The Amount of Calcium Oxalate Needles in Wet Conjuc Flour, Dry Conjuc Flour and Conjuc Soap

Based on the amount data of calcium oxalate in wet conjuc flour, dry conjuc flour, and conjuc soap. It shows that the highest number of calcium oxalate is in wet conjuc flour, the lower number of calcium oxalate is in dry conjuc flour, and there is no

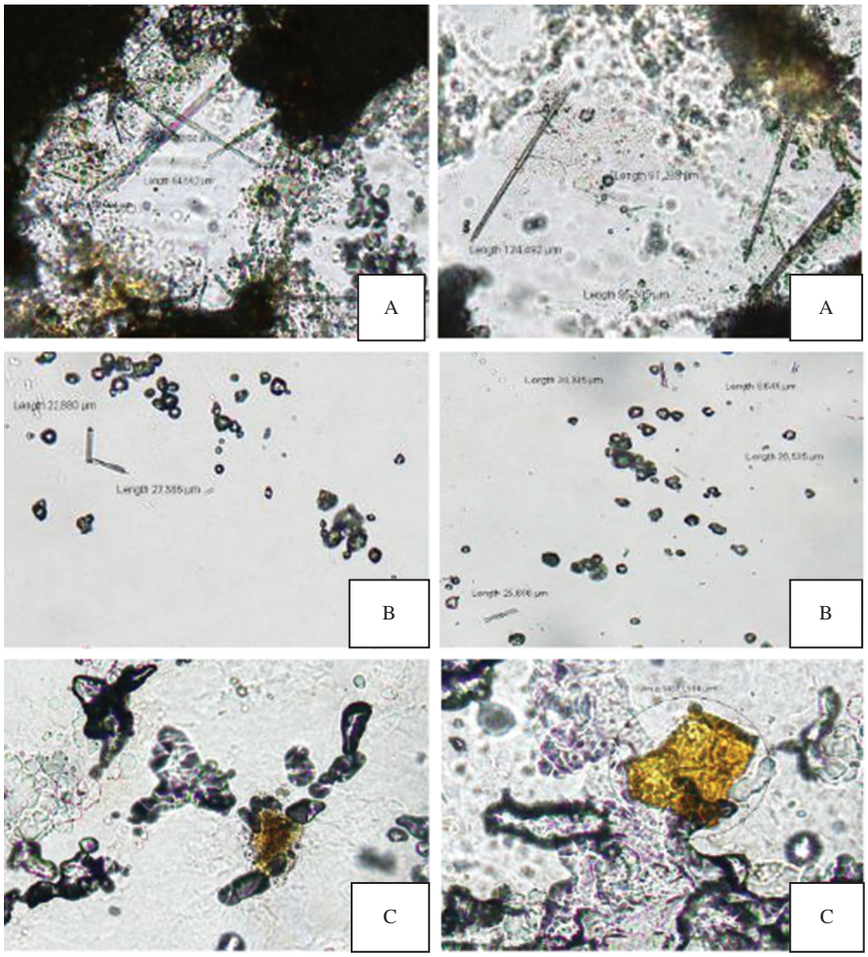

Fig. 2. Structure of calcium oxalate in (A) wet conjuc flour; (B) dry conjuc flour; (C) conjuc soap.

calcium oxalate in conjuc soap. However, ANOVA test shows that different ingredients (wet conjuc flour, dry conjuc flour, and conjuc soap) have no significant effect in the amount of calcium oxalate needles. The average number of calcium oxalate can be seen at Figure 3.

The high number of calcium oxalate needles that are found in wet conjuc flour because there is no sunbathing process and boiling process in the making process of wet conjuc flour which

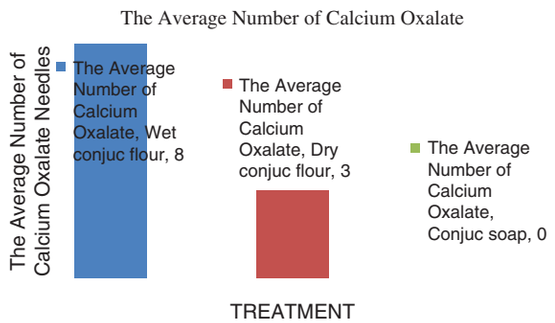

Fig. 3. The average number of calcium oxalate needles in wet conjuc flour, dry conjuc flour, and conjuc soap.

can reduce the high number of calcium oxalate needles. There is a significant difference between crude flour (control flour) and best treatment flour. Control flour has the higher number of calcium oxalate needles that best treatment flour and glucomannan flour. Control flour contains 22,7% calcium oxalate from 100 grams of control flour. While best treatment flour contains 3,23% calcium oxalate from 100 grams of best treatment flour and glucomannan flour contains 0,08% calcium oxalate from 100 grams of glucomannan flour. It shows that control flour has higher number of calcium oxalate than best treatment flour.[11]

Conjuc soap doesn't have calcium oxalate needle because there is boiling process in the making of conjuc soap that deprives oxalate needles. The number of calcium oxalate after boiled 60 minutes, it will reduce the number of calcium oxalate 55,91% at 30 °C and up to 93,1% at 60 °C. Boiling process can ruin cell wall that leads calcium oxalates leak out and it's soluble in the hot water.[12] The higher temperature can reduce more calcium oxalate.[13] Boiling process can degrade calcium oxalate into calcium carbonate and carbon monoxide as reaction below:

$$CaC_2O_{4(s)} + \text{heat} \rightarrow CaCO_{3(s)} + CO_{(g)}$$

Maximum heating until 60 °C will impair nutrient content in a tuber such as protein, carbohydrates, minerals, and vitamin.

Reducing calcium oxalate by cooking tubers lead to positive impact in human health. Cooked tuber can increase mineral absorption in the body and decrease the risk of kidney failure.[13]

3.2.3. Length of Calcium Oxalate in Wet Conjuc Flour, Dry Conjuc Flour, and Conjuc Soap

Based on length data of calcium oxalate in wet conjuc flour, dry conjuc flour, and conjuc soap, it shows that the longest size of oxalate needle is found in wet conjuc flour. However, ANOVA test shows that the difference in treatment ingredients (wet conjuc flour, dry conjuc flour and conjuc soap) have a real effect to the length of calcium oxalate needles. The results of further analysis test by Duncan test shows that there is significant difference between wet conjuc flour and conjuc soap, but there is no real difference between dry conjuc flour with wet conjuc flour and conjuc soap.

Reduction of calcium oxalate is caused by drying process in dry conjuc flour and boiling process in conjuc soap. Moreover, in process of drying and boiling, either in powder or soap, both of dry conjuc flour and conjuc soap, there is a homogenization process in blender that helps reduction process so that the structure of calcium oxalate needles falter even disappear.

Several methods of reducing the content of calcium oxalate (mg/100 g) shown by heating at temperature 90 °C during 40 minutes can reduce 70% of calcium oxalate. Tubers soaking in warm water at temperature 38–48 °C for less than 4 hours can reduce the level of component that causes itching without causing starch gelatinization.[14] The average length of the calcium oxalate needles in each treatment are presented in Figure 4.

Based on these data, it can be seen that the average length of the oxalate needles on dry conjuc flour is smaller than wet conjuc flour. That because the process of making dry conjuc flour uses drying step which causes calcium oxalate in porang is reduced and disjointed into smaller shapes. Heating the drying process can eliminate calcium oxalate in porang.[15]

The existence of the boiling process in the manufacturing of conjuc soap causes calcium oxalate disjointed and spread out even covered up is not visible or disappear. That because the boiling process potentially can damage the structural components of calcium oxalate. Calcium oxalate decreasing levels due to boiling related to oxalate solubility in water is actually increases at high temperatures treatment. Boiling process also causes the release of calcium oxalate in tubers into the boiling water.[12] Heating at temperature of 90 °C during 40 minutes can reduce the content of calcium oxalate up to 82,1%, while heating at temperature of 165 °C during 40–45 minutes can reduce calcium oxalate up to 46,6%. The changing in calcium oxalate rafida form of whole becomes discontinuous into short and blunt even as disappearing is due to chemical and mechanical process.[16]

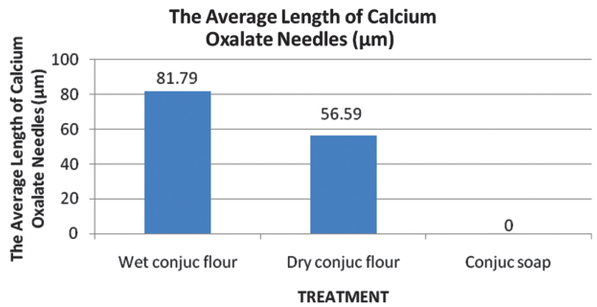

Fig. 4. Length of calcium oxalate in wet conjuc flour, dry conjuc flour, and conjuc soap.

4. CONCLUSION

Based on the results test of protein degradation ability shows that conjuc soap has the highest protein degradation ability and wet conjuc flour has the lowest protein degradation ability. This is because there is the boiling process in the making of conjuc soap that accelerates the protein degradation process. Structural change of calcium oxalate in wet conjuc flour, dry conjuc flour, and conjuc soap shows that wet conjuc flour contains the highest number of calcium oxalate and the longer size of calcium oxalate, dry conjuc soap contains lower number of calcium oxalate and shorter size of calcium oxalate and conjuc soap doesn't have calcium oxalate. Conjuc flour can be potentially processed into conjuc soap. It is based on the results of study shows that conjuc soap has the highest level of protein degradation ability and contains low level of calcium oxalate.

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