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Application of Chitosan Solution to Reduce Content Purine Substances (Total Alkaloids) in Melinjo Seeds

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A R T I C L E I N F O

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A B S T R A C T

Melinjo beans are one of the mainstay commodities of Indonesian society. One of the food products produced from melinjo beans is melinjo chips but inside melinjo beans there is a relatively large amount of purine substance. Consuming foods that contain a lot of purine (total alkaloids) can cause gout or swelling in the joints. This research aims to reduce the content of purine inside melinjo beans. The method to decrease total alkaloids by immersion process using water and chitosan solution. The various chitosan solution's concentrations were 0.5%, 1.0% and 1.5% at time 20,40 and 60 minutes and at temperatures 30oC, 50oC and 70oC. The analyzed for level of ash, total protein as well as purine content (total alkaloids). The results showed that purine content inside melinjo beans before immersion process was 1.3264% and after immersion had decreased. The lowest purine content after immersion with water solution was 0.1033%, at a concentration of 0.5% was 0.0899 %, at a concentration of 1.0% was 0.0878% and at a concentration of 1.5% was 0.0711%. The lowest total alkaloids were obtained at temperature 50˚C and at time 40 minutes with the decrease until 94.63%

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INTRODUCTION

Melinjo plants (Gnetum Gnemon L) can grow in almost every region in Indonesia. Almost all parts of the melinjo plant can be processed into products ranging from leaves, flowers, to melinjo fruit. Melinjo seeds can be processed into melinjo chips by boiling or roasting them, then flattening them and drying them in the sun. Emping melinjo is very popular with the public, but research results show that it contains quite a large amount of purine, namely 50-150 mg/100 grams (Munajad, 2009). Food is considered to have a low purine content if the content is less than 25 mg/100g of food (Ellington, 2005).

Consuming foods that contain excess purine can cause high levels of uric acid in the body because the remaining crystals of purine metabolism (total alkaloids) can cause gout which is characterized by swelling of the joints. Purine itself is an alkaloid compound that is easily soluble in water in the form of its salt (Padmawinata, 1995). Therefore, it is necessary to find a way to make emping melinjo products that are low in purine content (total alkaloids).

Based on several studies above, to reduce the purine content (total alkaloids) in emping melinjo, a soaking process has been carried out using a chitosan solution. Chitosan is able to adsorb and be used as a chelating agent, namely a substance that has two or more donor atoms that can bind chemical elements in very small concentrations (trace elements).

The chitosan solution was added with varying concentrations, times and temperatures. This treatment was carried out to determine the effect of adding chitosan solution on reducing the levels of purine substances (total alkaloids) in emping melinjo. It is hoped that emping melinjo products consumed by the public will not cause a high risk of developing gout.

Based on the background description above, the formulation and limitations of the problem that will be examined in this study are; (i) what is the reduction in purine substances (total alkaloids) in melinjo seeds soaked in chitosan solution at varying concentrations of 0% (control), 0.5%, 1.0%, and 1.5%?, (ii) what is the effect? immersion in chitosan solution at temperatures of 30oC, 50oC, and 70˚C on reducing purine levels in melinjo seeds?, and (iii) what is the effect of varying soaking times in chitosan solution for 20, 40, and 60 minutes in chitosan solution on reducing levels purine substances? It is hoped that the results of this research will produce melinjo seeds with low purine content (total alkaloids) and can be applied by melinjo emping producers.

LITERATURE REVIEW

Defriana Annisa (2014) conducted an experiment by soaking melinjo chips in water for 2 hours and was able to reduce the total level of purine bases by up to 50%, especially hypoxanthine. Research by Siti Salamah et al (2015) has succeeded in reducing purine substances in melinjo seeds using the blanching method, melinjo seeds are soaked in NaHCO3 solution and heated at a temperature of 80˚C. The optimum soaking time to reduce purine levels is 24 hours with a reduction of 32%. Cui Suping et. al (2012) has conducted research on chitosan solution which has been proven to be able to reduce the levels of purine substances in soybeans for making tofu from initially 253-275 mg/100 g to 123.5-130 mg/100 g.

METHODOLOGY

1. Making Chitosan Solution

The chitosan that will be used is chitosan and is dissolved in acetic acid solvent (CH3COOH) with a concentration of 0.5%, 1.0% and 1.5% (w/v) then stirred until homogeneous.

2. Soaking Melinjo Seeds in Chitosan Solution

Melinjo fruit to be used must be peeled, boiled and then pounded first to obtain melinjo seeds and then soaked in chitosan solution. The treatments given to the samples were variations in the concentration of adding water and chitosan solution (0.5%, 1.0% and 1.5%), variations in time (20, 40 and 60 minutes) and variations in temperature, namely mixing at a temperature (30oC , 50˚C, and 70oC).

3. Drying Process

The process of drying melinjo seeds can be done using direct sunlight or using tools that basically have the same principle. The basic principle of the drying process is that heat must be applied to the material and water must be removed from the material. The drying process using sunlight is carried out until the water content is a maximum of 12% (SNI 01-3712-1995). Samples are stored in a tightly closed place and analyzed.

Analysis Method

Several analyzes were carried out on the results of soaking samples of melinjo chips with chitosan solution, including:

a) Determination of Water Content

Determination of water content was carried out using the oven drying method (AOAC, 1995).

b) Determination of Total Prote in Content

Determination of the total protein content of emping melinjo was carried out using the Kjeldhal method (AOAC, 2001).

c) Determination of Total Alkaloid Content

Melinjo fruit samples were peeled and then boiled for 1 hour. The melinjo fruit has been boiled and then mashed until flat melinjo seeds are obtained and blended until it becomes fine melinjo seed flour.

Extraction Process (Saifudin, 2011)

- Weigh 320 grams of ground melinjo flour.
- Then put it in a sterile Erlenmeyer flask and add 96% ethanol solution until it is completely submerged ± 300 ml.
- Stir so that the sample becomes homogeneous then cover and wrap the surface of the container using aluminum foil.
- Next, put it in the incubator shaker for 3x24 hours at room temperature, closed and protected from direct light.
- Then filter the resulting macerate using filter paper to separate solids and liquids.
- The total macerate obtained is then put in a rotary evaporator at a temperature of 40˚C until it becomes Crude Crystal.
- The crude crystal is then weighed and the yield is calculated.

Total Alkaloid Test (Phytochemical Methods. Harbone, 1973)
- Weigh 2.5 grams of crude crystal and dissolve it in 50 ml of 10% acetic acid solution (in ethanol)
- The solution was homogenized with a magnetic stirrer for 4 hours
- The filtrate is then evaporated, and dripped with ammonium hydroxide until no alkaloid precipitates occur.
- First weigh the filter paper that will be used to filter the sediment
- Then the precipitate is filtered and washed using 1% ammonium hydroxide solution
- The filter paper containing the precipitate is dried in an oven at 60˚C for 30 minutes.
- After cooling, the precipitate is weighed until a constant weight is obtained.
d) Ash Content Test
Determination of ash content using the gravimetric method (AOAC, 2005)

RESULTS AND DISCUSSION

Initial Composition of Melinjo Seeds

Melinjo seeds are the raw material used to make melinjo chips. The chemical ingredients contained in melinjo include carbohydrates, protein, fat, starch, phenol, calcium, phosphorus, iron, vitamin A, vitamin B and several alkaloids, flavonoids, saponins and tannins. Analysis of water content, ash content, protein content and purine content (total alkaloids) is carried out before the soaking process at varying concentrations, time and temperature. The results of the analysis can be seen in the following table.

Table 1. Results of Melinjo Seed Composition Analysis

Chemical content	%
Water content	23,28
Ash content	1,93
Protein Content	9,98
Total Alkaloid Content	1,32
Etc	63,49

The chemical composition of melinjo seeds varies depending on the climate, soil conditions, place of growth and the degree of maturity of the melinjo seeds (Directorate of Nutrition of the Indonesian Ministry of Health, 1998). One of the chemical compounds contained in melinjo seeds is alkaloids (purine substances). Purines are included in the group of nitrogen bases which have derivative compounds, namely hypoxanthine, xanthine, theobromine, caffeine and isoguanine. According to Rodwell (2003) Purine consumed by the body will be converted into hypoxanthine by the xanthine oxidase enzyme and converted into xanthine which is then oxidized into uric acid.

The chemical composition that determines the quality of melinjo seeds is water content. Good quality melinjo seeds are those that are large, old and

contain a small amount of water so that when processed into emping they will not experience heavy shrinkage (Adiatama, 1995). The water content obtained in the initial melinjo seeds was 23.28%. This water content is still high when compared with SNI 01-3712-1995 which states that the maximum water content requirement for emping melinjo is 12%. This high water content is caused by the method used to dry the sample using the simplest and most effective drying method, namely using sunlight, so it is necessary to dry it again until the water content obtained is in accordance with SNI.

Results of Analysis of Changes in Purine Content (Total Alkaloids) in Chitosan Solution Soaking

The purine substances (total alkaloids) contained in melinjo seeds are the main cause of gout or gout. Gout is a type of arthritis caused by the buildup of crystals due to high levels of purine in the body. Analysis of total alkaloid content (purine substances) in this study used maceration and gravimetric methods because this method is simpler, does not require special equipment and heating so it can overcome the possibility of the presence of decomposing compounds. In general, total alkaloids can be dissolved in their salt form. The decrease in purine levels (total alkaloids) can be seen in the following pictures,

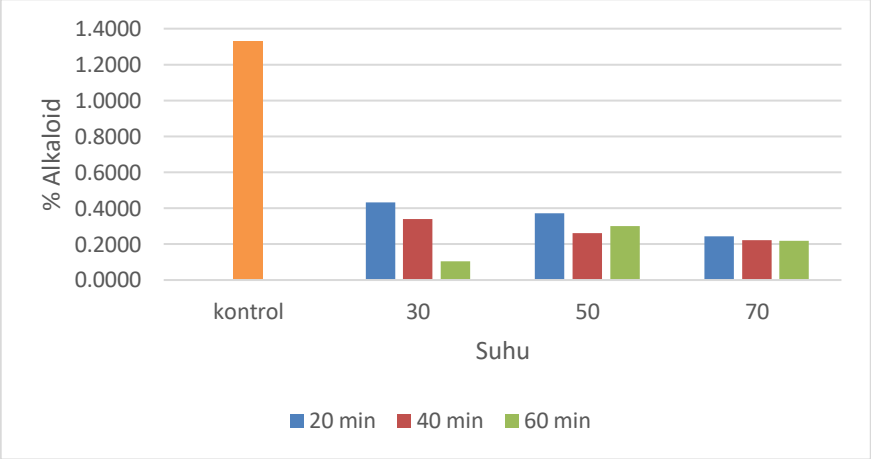

Figure 1. Results of Total Alkaloid Analysis When Soaked in Water

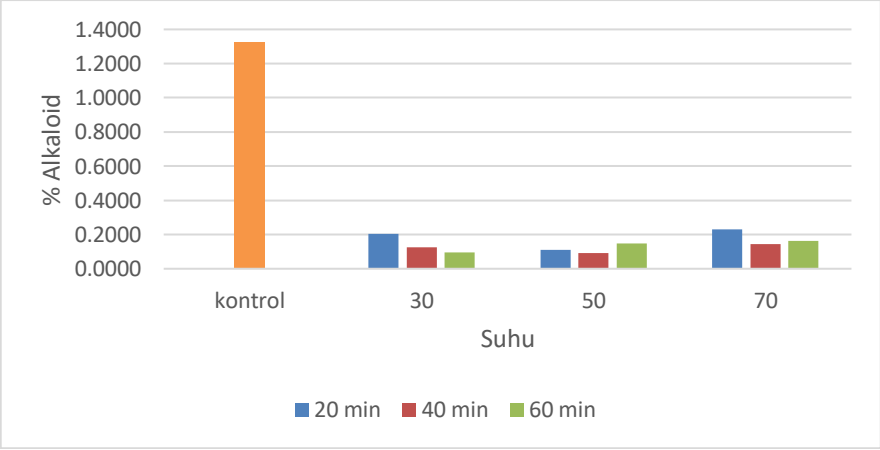

Figure 2. Results of Total Alkaloid Analysis When Immersing Chitosan at a Concentration Of 0.5%

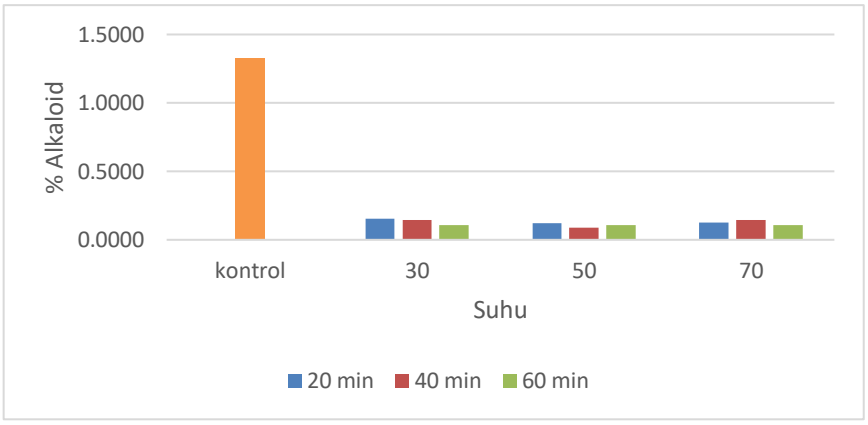

Figure 3. Results of Total Alkaloid Analysis When Immersing Chitosan at a Concentration of 1.0%

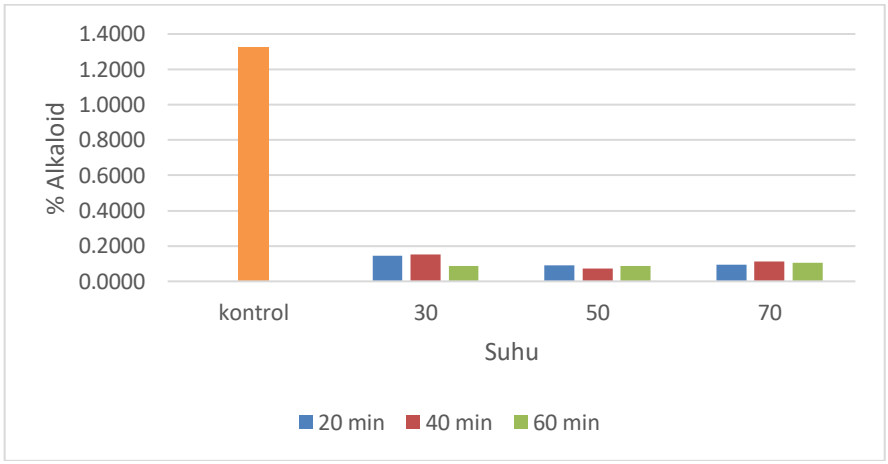

Figure 4. Results of Total Alkaloid Analysis at 1.5% Concentration Immersion

The alkaloid levels in all graphs prove that the large concentration of chitosan solution influences the reduction in total alkaloid levels with a value of 0.0899% - 0.2309% for a 0.5% chitosan solution concentration, at a concentration of 1.0% the total alkaloid content value obtained is 0.0878% - 0.1529% and at a concentration of 1.5% the total alkaloid content reached the lowest point, namely 0.0711% and the highest was 0.1525%. The alkaloid content in the control was 1.3264% and the lowest alkaloid value after soaking was 0.0711% so that the decrease in purine content (total alkaloid) was 94.63%. This is due to the influence of one of the properties of chitosan which can be used as an adsorbent. Not only does it absorb alkaloids, but chitosan is able to absorb heavy metals such as Zn, Cd, Cu, Pb, Mg and Fe according to Knoor (1984). Chitosan also has high chemical reactivity because it is supported by the polar and non-polar groups it contains and causes chitosan to be able to attract molecules such as oil, fat, protein and others. Apart from that, chitosan is also known as a chelating agent, namely a substance that has two or more donor atoms that can bind chemical elements in very small concentrations (trace elements). Another factor that causes purine substances (total alkaloids) to be reduced is the use of acetic acid (CH3COOH) to dissolve chitosan. Purine substances (total alkaloids) are compounds that are basic and can dissolve in water in the form of salts. The purine salting process can be carried out using acetic acid and almost all acetate salts dissolve well in

water. Latifaningsih (2012) reported that the longer the soaking time and the higher the concentration of acetic acid could reduce the total alkaloid content.

The temperature of soaking melinjo seeds in chitosan solution does not really affect the reduction in purine levels (total alkaloids) and tends to fluctuate. This can be seen from the value of alkaloid levels at a temperature of 30 oC which tends to decrease at a temperature of 50oC but increases again at a temperature of 70oC. This unstable change can occur because soaking the sample has reached the optimum temperature to reduce purine levels (total alkaloids). Rianta Pratiwi (2014) stated that chitosan that is stored for a long period of time at temperatures above 100 ^{o}F can experience changes in its overall properties and viscosity. Then the total alkaloid results from varying soaking times of 20 minutes, 40 minutes and 60 minutes tended to be unstable and increased at 60 minutes. The decrease in alkaloid levels occurs because during the soaking time chitosan can adsorb total alkaloids (purine substances) from its complex compounds. The level of purine substances (total alkaloids) which actually increased again after 60 minutes could occur because the chitosan solution used to soak the melinjo seeds was already in a saturated state.

CONCLUSIONS AND RECOMMENDATIONS

The conclusions obtained from research on the application of chitosan solution to reduce the purine content in melinjo seeds are:

1) The lowest total alkaloid content after soaking was obtained at a 1.5% chitosan solution concentration of 0.0711%.

2) The decrease in purine levels (total alkaloids) in melinjo seeds at temperature and soaking time tends to fluctuate. The lowest total alkaloid content at a temperature of 30 for 60 minutes was 0.0857%, a temperature of 50oC for 40 minutes was 0.0711% and a temperature of 70oC was 0.0937%. The lowest alkaloid content was produced by soaking for 20 minutes at 0.0904%.

3) The lowest levels of purine substances (total alkaloids) were obtained when soaking at a temperature of 50oC and a time of 40 minutes with a reduction of up to 94.63%.

4) It is necessary to carry out a method for testing specific purine substances, using a more sophisticated method such as using an HPLC tool so that the specific purine content that causes gout can be detected thoroughly.

FURTHER STUDY

This research still has limitations, so it is necessary to carry out further research related to the topic of Application of Chitosan Solution to Reduce Content Purine Substances (Total Alkaloids) in order to improve this research and add insight to readers

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