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EFFECT OF FERMENTATION TIME ON MINERAL PROFILE AND TOTAL MOLD OF COWPEA (Vigna unguiculata) TEMPEH

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ABSTRACT

Tempe is one of the popular foods in Indonesia which comes from fermented soybeans with the help of Rhizopus oligosporus. The development of tempeh with non-soy ingredients is starting to be carried out frequently. Cowpea (Vigna unguiculata subsp. unguiculata) is one of the nuts that is often consumed by Indonesian people. Cowpeas can be an alternative substrate for tempeh fermentation, because of their nutritional content, including vitamins and minerals, which are quite good for the body. However, some of these minerals are still bound to the phytic acid compound so it needs to be explained by the fermentation process. This research aimed to determine the ash content, mineral content of iron, calcium, and total mold in cowpea tempeh at a fermentation time of 35, 45, and 54 hours using standard methods. The research design used was a Completely Randomized Design (CRD) with repetition 3 times for each treatment for fermentation duration P1 (35 hours), P2 (45 hours), and P3 (54 hours). The ash and calcium content of cowpea tempeh decreased with fermentation times of 45 and 54 hours compared to 35 hours. In terms of iron content, the amount was almost the same at all fermentation times. Meanwhile, the total number of molds increased with fermentation times of 45 and 54 hours. Based on the results of observations for further research, the fermentation time for making cowpea tempeh used in this optimization was determined to be 35 hours. A fermentation time of 35 hours produces good cowpea tempeh, high calcium content, and efficient time and energy.

Key words: Cowpeas tempeh, Fermentation, Mineral profile, Total mold

INTRODUCTION

Tempeh is one of the most popular foods in Indonesia. Generally, tempeh is a fermented product of soybeans using Rhizopus oligosporus. This food is believed to have a protein-rich content that can be equal to animal protein. This taste is quite popular so that it is often chosen as a side dish or a daily snack. Tempeh is often referred to as the food of the future because of the various benefits that can be obtained such as preventing chronic diseases, diarrhea, improving brain cognitive function, preventing bone damage, and other benefits. (Bavia *et al*., 2012; Handajani *et al.,* 2021).

In recent years, non-soy-based tempeh has been developed. Researchers chose cowpea as one of the options to replace soybeans to be made into tempeh, considering that cowpea has a variety of benefits and content that is good for the body. Research of Saputro *et al* (2015) stated that the negative effects of using fatty food products can be minimized by consuming cowpeas, because it has low fat content. The process of making cowpea tempeh is no different from making tempeh in general, only by replacing soybeans with cowpeas. There are 3 factors to make a good tempeh product: (1). Cleanliness factor at every stage to prevent contamination; (2). The process of draining the seeds after boiling before fermentation, draining is important to inhibit unwanted microbial growth; (3). Temperature control during the tempeh curing process at (30-35 °C). (Bavia *et al*., 2012)

Cowpea (*Vigna unguiculata subsp. unguiculata*) is a legume plant that is quite often consumed by the community. Apart from being called “tunggak”, this bean is also known as tolo bean, dadap bean, and others. This bean is often used as food for the community. Cowpea seed extract can inhibit the growth rate of pathogenic fungi and bacteria (Jayathilake *et al.,* 2018). Other research shows that cowpea extract can act as an antidiabetic, anti-inflammatory, anticancer, antihypertensive, and antihyperlipidemic (Budianti, 2018). Cowpea has a protein content of 22-30%, carbohydrates of 33-59.9%, and crude fiber of 2.10-2.98% (Budianti, 2018). Another study showed that 100 g of mature cowpea seeds contain protein 22 g; fat 1.4 g; carbohydrates 51 g; water 10 g; vitamins 3.7 g; carbon 3.7 g; calcium 104 mg and other nutrients. The energy produced is 1420 kj/100 g or 339 kcal/100 g (Fadillah *et al.,* 2020).

Minerals are essential factors in food that cannot be produced by the body, and are inorganic elements that come from nature (Damayanti, 2016). The importance of knowing the mineral content in tempeh because of its excellent benefits for the body, one of which is by consuming tempeh regularly will prevent a person from anemia due to iron deficiency (Astawan, 2013). So the levels of macro and micro minerals found in tempeh can meet daily needs. Tempeh contains a number of minerals such as: zinc, copper and iron (Astawan, 2013). The molds in tempeh are able to produce phytase enzymes that break down phytic acid, which binds some minerals, into inositol and phosphorus. The breakdown of phytic acid allows certain minerals such as magnesium, iron, calcium and zinc to be more readily utilized by the body (Tamang *et al.,* 2016). Ash content shows the mixture of inorganic or mineral substances contained in foodstuffs (90% inorganic and water, the rest are mineral elements). Ash content can be used as an indicator of the total minerals in a food. In the combustion process, organic matter will burn, but inorganic components will not (Tamang *et al.,* 2016). The purpose of this study was to determine the mineral content and total mold of cowpea tempeh at 35, 45 and 54 hours of fermentation. The range of fermentation time is in connection with Dewi's research in 2014, related to the effect of fermentation time on the physico-chemical properties of cowpea tempeh with the highest score lies in the fermentation time of 36 hours. Not only that, the optimum increase in free amino acids in tempeh occurred after 48 hours of fermentation (Kanetro, 2017).

METHODOLOGY

Raw Materials

The materials needed include cowpea (Vigna unguiculata), RAPRIMA tempe yeast, agar media, acid solution, HCl, lanthanum chloride, ion-free distilled water, and Whatman filter paper.

Tools

The tools needed in this research include knives, cutting boards, scales, food dehydrator, grinder, vacuum sealer, 80 mesh sieve, spectrophotometer, vaporizer cup, analytical balance, oven, desiccator, test tubes, petri dishes, spreaders, porcelain chairs.

Research Design

The experimental design used was a Completely Randomized Design (CRD) with 3 treatments of different lengths of fermentation 35, 45, 54 hours (P1, P2, P3) each treatment was repeated 3 times. Statistical analysis of data using variance analysis and if there is a significant difference, it is continued with Duncan Mutiple Range Test (DMRT) at the 5% level. Processing of research data using the statistical program SA5.

Research Stages

1. Preparation of Cowpea Tempeh

The processing of cowpea tempeh and flattening was carried out at the Food Implementation Laboratory, Department of Nutrition, Faculty of Health Sciences, Universitas Brawijaya for two days. The cleaned cowpeas were then boiled for 15 minutes and cooled. After cooling, the cowpeas were then given tempeh yeast, put into banana leaves and covered then left for 35, 45 and 54 hours (P1, P2 and P3) for the fermentation process. After fermentation, cowpea tempeh was observed and preparations were made for making cowpea tempeh flour.

2. Preparation of Cowpea Tempeh Flour

The finished cowpea tempeh is chopped into small pieces to speed up the drying process. The cowpea tempeh pieces are then put into a food dehydrator at 60 °C for 1.5 hours to dry. After the tempeh is dry, it is pulverized into flour using a grinder and sieved using an 80 mesh sieve and cowpea tempeh flour is obtained. The finished flour was then put into plastic and vacuumed and stored for laboratory testing.

Methods

The tests carried out included Fe and Ca content tests using the Atomic Absorption Spectrophotometry (AAS) method, ash tests using the Gravimetric method, and total mold tests using the Spread Plate method.

Analysis Procedure

1. Determination of Ash Content by Dry Method (Sudarmadji *et al.,* 2010)

Porcelain curs with lid is heated in a muffle furnace. Before being put into a desiccator, it is cooled first in the oven and then weighed. Weighing the sample was done on a porcelain chair of known weight (2g), after which it was heated so that the material became charcoal and ignited in a muffle with a temperature of 600 °C for 6 hours until the sample became ash with a whitish color. The muffle was left to room temperature before opening the lid. The curs were placed in a dexicator to cool and weighed.

2. Mineral Content Measurement Using Atomic Absorption Spectrophotometer (AAS) Method (Sudarmadji *et al.,* 2010)

This method measures calcium and iron levels in cowpea tempeh. The residue from the removal of organic matter is dissolved in dilute acid. The solution is spread with the ignition contained in the AAS device until the metal emission or absorption can be measured and analyzed at a predetermined wavelength. The filter paper was cleaned with 3N HCl to remove tracemetal. Standard stock solution of 1000 mg/L by diluting 25 ml of 3N HCl to distilled water to 250 ml. If the sample is wet-fired, the standard solution is obtained from diluting the standard stock solution with water or if dry-fired, 0.3N HCL until the concentration is in the working range of the metal being measured. A total of 6 ml of 6N HCl was added to the cup containing the ash, then heated. 15 ml of 3N HCl was added to the cup and heated to boiling. The sample was then cooled and filtered using filter paper, the filtrate was put into a measuring flask. A total of 10 mL of 3N HCl was added to the cup, heated to boiling. The sample was then cooled and filtered using filter paper, the filtrate was put into a measuring flask. The cup was washed with water 3 times, the washing water was filtered and put into a measuring flask. Calcium content was determined by adding 5 mL of lanthanum chloride solution to each 100 ml solution. The mixture was cooled and diluted. A blank was prepared using the same number of reagents and the same steps. Then calibration of the equipment and sample determination were carried out.

3. Measurement of total mold by Spread plate method (Ptiwari and Tewari, 2009)

Preparation of PDA media where PDA powder (Merck) was weighed as much as 36.5 g in a glass cup, then 1 liter of distilled water was added. The media was heated to boiling on a hotplate and stirred using a stirrer. The boiling PDA media was then sterilized by autoclaving at 121 °C for 15 minutes. Isolates in test tubes were added with sterile 0.05% tween and spores were taken with an eyed ose until a spore suspension was obtained. The spore suspension was then diluted by taking 1 ml and putting it into 0.05% tween as much as 10 ml (fp: 10-1). Dilution is continued until the dilution factor is 10-4. Inoculation of fungi on PDA media. The growth of fungal mycelia on PDA media was observed daily by photographing from the top and side fields of view of the petri dish. Observation of fungal mycelia growth in the agar and spore formation was done by looking directly with a 100x magnification microscope.

RESULT AND DISCUSSION

Mineral profile and total mold in cowpea seeds and cowpea tempeh, consisting of ash content, iron content, calcium content and total mold can be seen in Table 1. From the three treatments, the most effective results were selected in optimizing the mineral content and total mold of cowpea tempeh. To determine the stability of the nutritional value of the cowpea tempeh product produced, chemical properties (ash content, iron/Fe content, calcium content according to SNI, and total mold in the product were tested. Based on the results of observations for further research, it was determined that the length of fermentation in making cowpea tempeh used in this optimization was 35 hours. The fermentation time of 35 hours produced good cowpea tempeh, high calcium content, and efficient time and energy.

Table 1. Mineral Content and Total Mold of Cowpea Tempeh

Component	Cowpea	P1	P2	P3	*Standard (SNI)
Ash content (%)	2.51	3.53^{b}	1.79^{a}	1.52^{a}	Max 1.5
Fe Content (ppm)	6.5	30.04^{a}	30.14^{a}	30.02^{a}	Min 4
Ca content (ppm)	77	188.32^{b}	88.95^{a}	85.24^{a}	Min 155
Total Mold (CFU/g)	-	$3.8x10^{6a}$	$1.8x10^{7b}$	$1.6x10^{7b}$	-

Description:
35 hours cowpea tempeh, (P1), 45 hours cowpea tempeh (P2), 54 hours cowpea tempeh (P3). Different letter superscript notation in each column mean indicates significantly different (p<0.0)

1. Ash Content

Ash is one of the inorganic substances left over from the combustion of foodstuffs. The ash content and its component materials depend on the method of ignition and the materials used. Ash content is related to the minerals of a material, which generally consist of organic and inorganic salts. There are about 96% organic matter and water in most foodstuffs, the rest is an element of ash content (minerals) (Mukhtadi *et al.,* 1993; Tamang *et al.,* 2016). The presence of minerals in cowpea is utilized by molds as nutrients to grow so that it has good cohesiveness. Optimal metabolism is indicated by the compactness of the mycelium due to the activity of Rhizopus oligosporus (Fadillah *et al.,* 2020). The results of ash content analysis of cowpea seeds and cowpea tempeh with fermentation duration (35 hours, 45 and 54 hours) can be seen in Table 1.

The difference in fermentation duration affects the ash content of cowpea tempeh. The longer the fermentation time, the ash content of cowpea tempeh will decrease at 45 hours of fermentation. This is in line with the research of Fauziah *et al* in 2022, which states that the longer the fermentation time, the ash content decreases. The highest ash content in cowpea tempeh with 35 hours of fermentation (P1) is (3.53%) compared to 45 and 54 hours of fermentation. The ash content of cowpea tempeh ranged from 1.6 - 3.98%, indicating that the amount of mineral content in cowpea tempeh was higher than the ash content of soybean tempeh (1.3%) (Jannah, 2011). The high ash content of cowpea tempeh is due to the total mineral composition in cowpea such as calcium, phosphorus, and iron.

The results of cowpea tempeh fermentation such as vitamin B12 affect the increase in ash content formed by bacterial growth during the fermentation process (Winarno, 2002). Vitamin B12 is produced by Klebsiella pneumoniae which synthesizes sucrose found in carbohydrates as a food source. Vitamin B12 contains one atom of Cobalt (Co) bound to iron-like molecules in hemoglobin or magnesium in chlorophyll (Mukhtadi *et al.,* 1993), so the increase in ash content comes from Cobalt that occurs during the fermentation process of cowpea tempeh.

In this study, the ash content of cowpea tempeh decreased when compared to the ash content of cowpea seeds, this can occur due to hydrolysis during soaking. Minerals contained in cowpea seeds will be dissolved in water during soaking, besides that boiling plays a role in reducing mineral content. According to SNI, the maximum ash content in tempeh is around 1.5%. The concentration of ash content is related to the minerals in a material, if the ash content in the material decreases, the mineral content of the material also follows.

2. Calcium Content

Cowpea has a high calcium content of 481 mg/100g of material compared to cow's milk which contains about 143 mg/100g, mung beans 223 mg/100g and soybeans 222 mg/100g. With fermentation, the mineral content in cowpea tempeh will decompose and increase, one of which is calcium. The results of calcium content analysis with the length of fermentation in cowpea tempeh can be seen in Table 1. In the calcium content of cowpea tempeh with 35 hours of fermentation (P1), the value is higher (188.32 ppm) compared to 45 and 54 hours of fermentation. There was a decrease in calcium content after fermentation for 35 hours. This is in line with research on changes in vitamin and mineral levels in fermented cowpea tempeh, where calcium levels decreased by 52.72% after the fermentation process (Sine *et al.,* 2018).

Calcium levels in cowpea tempeh are influenced by the activity of tempeh yeast in the process of reducing phytic acid in raw seeds before fermentation. This is caused by the phytase enzyme that breaks down phytic acid into inorganic phosphorus and inositol (Jannah, 2011). This breakdown causes minerals such as calcium to become available in tempeh to be more easily absorbed by the body. In addition, the decrease in phytate levels was influenced by the treatment of yeast levels given and the length of fermentation. The higher the concentration of yeast added, the more enzymes are produced during the fermentation process to break down nutrients and minerals. The amount of phytic acid released will increase according to the length of the fermentation process (Fadillah *et al.,* 2020).

3. Fe (Iron) Content

The fermentation process for making cowpea tempeh will increase the availability of iron from materials in non-heme form (Ratnaningsih *et al.,* 2009). The form of iron in tempeh is ferric ($Fe3+$) which if absorbed in the body will be in the form of ferrous ($Fe2+$). The average iron content in tempeh without fortification is 2.0 mg (Novianti *et al.,* 2019). The results of the analysis of iron content with the length of fermentation in cowpea tempeh can be seen in Table 1. For Fe content, the value is almost the same in P1 (30.04 ppm), P2 (30.14 ppm) and P3 (30.02 ppm). These results are supported by a study conducted by Novianti et al (2019), which states that the enzyme activity produced by Rhizopus oligosporus fungus can increase iron solubility from 24.3% in raw soybeans to 40.5% in tempeh.

4. Total mold

The mold found in tempeh is able to produce lipase, protease, and amylase enzymes that have a function in the process of breaking down proteins, fats, and complex carbohydrates into simpler forms of substance (Astuti *et al.,* 2014). The amino acids contained in tempeh are 24 times higher than soy milk so tempeh is very good for consumption by all ages. The fermentation process also helps increase the concentration of folic acid and produce vitamin B12 from bacteria that are not found in other vegetable products (Novianti *et al.,* 2019).

Tempeh molds are able to produce phytase enzymes that function to break down phytic acid (which binds several types of minerals) into inositol and phosphot. This decomposition makes certain minerals, such as iron, calcium, magnesium, and zinc, more available to be utilized by the body. The soaking process is also responsible for the increase in phytase enzymes, thus reducing the anti-nutrients in cowpea seeds. Phytic acid is water-soluble, thus reducing the relatively large concentration of phytic acid during the soaking process.

Based on Table 1, it is known that the highest total mold value is obtained in P2 and P3 (1.8.107 CFU/g and 1.6.107 CFU/g) while P1 has the lowest value (3.8.106 CFU/g) (looking for references). In line with the results of research showing the number of yeast concentrations that are higher with the longer fermentation time gives the results of tempeh with a surface covered with mold mycelium that is evenly distributed and compact and white in color (Sofiyatin *et al.,* 2015).

Rhizopus oligosporus mold can increase the nutritional value of tempeh and inhibit the growth rate of pathogenic bacteria. The tempeh fermentation process can be divided into 3 phases, namely: (1) rapid growth phase (0-30 hours of fermentation), where there is an increase in free fatty acid levels, an increase in temperature, rapid growth of molds and forming mycelium on the surface of soybean seeds to produce a denser mass; (2) transition phase (30-50 hours of fermentation), which is the optimal phase of tempeh fermentation ready for market. In this phase there is a liberation of fatty acids, a decrease in temperature, and a small amount of mold growth, optimal tempeh specific aroma and a more compact texture; (3) advanced fermentation or decay phase (50-90 hours of fermentation) where there is an increase in the concentration of bacteria and free fatty acids, mold development decreases at certain water concentrations, changes in aroma and protein degradation to form ammonia (Radiati & Sumarto, 2016). Optimum cowpea tempeh fermentation is carried out at a temperature of 25-37oC for 36-48 hours (Eliyana, 2017). The longer the fermentation process occurs, the higher the total mold contained in it to break down compounds into nutrients that are more easily digested by the body. In addition, the higher the total mold, the more compact the resulting tempeh shape (Hayati, 2010; Kasmidjo, 1989; Rahmawati & Suntornsuk, 2016).

The mold will hydrolyze complex compounds into simpler ones during fermentation, resulting in an increase in the degree of unsaturation of fatty acids in tempeh. In addition, the availability of protein in dissolved form, increased production of B vitamins, and increased availability of certain minerals such as calcium, iron, magnesium and zinc, as well as producing the distinctive taste and aroma of tempeh (Sine Yuni & Soetarto, 2018). This indicates that tempeh has good nutritional quality due to the content of protein compounds, vitamins and minerals needed by the body.

CONCLUSION

Based on the results, the mineral characteristics of cowpea tempeh, ash content and calcium content, decreased in 45 and 54 hours of fermentation compared to 35 hours of tempeh fermentation. For Fe content, the value was almost the same in all three fermentation lengths (35, 45, and 54 hours). Meanwhile, the total mold in cowpea tempeh with 45 and 54 hours of fermentation treatment had a higher value compared to 35 hours of fermentation. In addition to the length of fermentation, the value is influenced by the suitability of oxygen levels, temperature, and humidity of the packaging device when tempeh fermentation takes place. Cowpea tempeh has a higher digestibility for the body, this is due to the hydrolysis of anti-nutritional compounds in cowpea such as tannins and trypsin inhibitors during the tempeh making process. Phytase enzyme produced by microorganisms active in the fermentation process is able to break down phytic acid into inositol and phosphorus.

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