



Effects of Pretreatment and Solid-State Fermentation on *Tempeh* Protein Content

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ABSTRACT

In this study the effect of pretreatment and solid-state fermentation (SSF) on *Tempeh* protein content has been studied. Pretreatment and SSF are important processes for producing high-quality *Tempeh*. Based on the results of the analysis using a UV-Vis spectrophotometer at $\lambda_{\text{max}} = 540 \text{ nm}$, it is known that both pretreatment and SSF cause the reduced *Tempeh* protein content. Pretreatment causes the total protein content (w/w) to decrease rapidly from 1.05% to 0.82%, while the SSF causes the content dissolved protein (w/w) reduced from 0.57% to 0.11%. In addition, SSF increased the *Tempeh* pH from 4.67 to 5.65 and decreased the water content (%) from 6.38 to 2.04.

Keywords: *Tempeh*, Pretreatment, Solid-state fermentation, Protein content.

INTRODUCTION

Tempeh is a traditional Indonesian food that is produced through the process of soybean fermentation using *Rhizopus* sp^{1,2,3}. *Tempeh* attracts many people not only in Indonesia, but also at this time it is reportedly consumed by people in several countries. *Tempeh* has a special taste and several benefits to the human body have been reported such as inhibiting the biosynthesis of cholesterol

in the liver, preventing LDL oxidation, increasing the antioxidant SOD enzyme, reducing the risk of rectal, prostate and breast cancer, reducing total cholesterol and triacylglycerol^{4,5}.

The use of soybeans as the main raw material for making *Tempeh* is based on its high protein content composed of various types of amino acids⁶. Protein is reported as an important parameter in explaining the quality of *Tempeh*. In this case, the



Indonesian National Standardization Agency (BSN) No. 3144 of 2012 stipulates that the minimum protein content in *Tempeh* is 16% (w/w)⁷. In the acquisition of protein levels, the manufacturing process becomes a determining factor in the availability of protein⁸. In general the process of making *Tempeh* consists of two important stages, namely raw material pretreatment and fermentation. Several stages of pretreatment are carried out during the process of making soybean *Tempeh* including boiling, grinding, washing, soaking, and steaming. In the boiling and steaming stages, it is usually done at high temperature conditions so that it will have an impact on the availability of protein⁹. As with the pretreatment process, the fermentation process is also reported to affect the availability of the amount of protein¹⁰. The presence of protease enzymes formed during the fermentation process will gradually denature proteins¹¹.

This study was conducted to determine the effect of the pretreatment and solid-state fermentation (SSF) processes on the amount of *Tempeh* protein. Although research on *Tempeh* has been widely reported, it has not specifically reported the effect of the manufacturing process on the content of tempeh protein. Information about changes in protein content during the process of making *Tempeh* needs to be known, because in the last year *Tempeh* was not only consumed directly by the community, but tempeh was also used as an alternative food additive to increase the nutritional value of coconut milk sauce¹².

MATERIALS AND METHODS

Making *Tempeh*

Soybeans washed and then boiled for ± 60 minutes. Furthermore, soaked for ± 720 minutes. After soaking, the soybean epidermis is peeled and washed then steamed for 45 minutes. The soybean seeds are then drained, after being cold added *Rhizopus sp.* Where every 2 g *Rhizopus sp.* homogeneously mixed into 1 kg of soy beans. Furthermore, it is wrapped in plastic and incubated at room temperature for 1 day, 2 days, 3 days, 4 days, and 5 days, respectively.

Analysis of protein content

Sample preparation

Tempeh with different fermentation times (1-5 days) is dried in an oven at 80°C for 24 hours. then mashed and filtered using a sieve with a pore size of ± 0.3 mm.

Total Protein Levels

A total of 0.50 g of the tempeh was added to the destruction flask and 10 mL of concentrated H₂SO₄ was added. Then it is destructed until all the samples in the destruction flask dissolve and the liquid becomes clear greenish. After the digestion process is complete, the solution is left for a while to cool. Furthermore as much as 1.0 mL of the digestive solution was taken and the biuret reagent was added as much as 3.0 mL. Then stirred at vortex and incubated at room temperature for 30 minutes. Then absorbance was measured at a maximum wavelength (λ_{max}) of 540 nm using a UV-Vis spectrophotometer.

Dissolved Protein Levels

A total of 1.0 g of the *Tempeh* was dissolved with 50 mL phosphate buffer pH 8 and stirred with a magnetic stirrer. Then the mixture was centrifuged and filtered using filter paper. The obtained filtrate was transferred into a 50 mL measuring flask, then a phosphate buffer pH 8 was added to the limit mark. The filtrate was taken 1.0 mL, then a 3.0 mL biuret reagent was added. Stir in the vortex and incubate at room temperature for 30 minutes. Then the absorbance was measured at the maximum wavelength (λ_{max}) of 540 nm using a UV-Vis spectrophotometer.

pH analysis

A total of 5.0 g of the *Tempeh* was mixed with 10 mL of distilled water (comparison of sample and distilled water ie 1:2). The mixture was then stirred using a magnetic stirrer for 30 minutes. The pH of the subsequent sample was measured using a pH meter.

Water content analysis

A total of 2.0 g of the *Tempeh* is dried in the oven for 1 hour. Cooled in a desiccator, then weighed dry weight. The treatment is repeated until a constant weight is obtained, then the water content is calculated.

$$\text{Water content} = \frac{(w1 - w2)}{w2} \times 100$$

Notes:

w1 = Weight of sample before drying (g)

w2 = Weight of sample after drying (g)

RESULTS AND DISCUSSION

Effect of pretreatment of *tempeh* raw materials

Figure 1A shows the effect of the

pretreatment process on both total and dissolved soy protein content. Pretreatment-1 is a process of boiling, grinding, washing and soaking. While pretreatment-2 is a steaming process, this process is the final process of soybean pretreatment before the addition of *Rhizopus sp.* The final pretreatment process generally causes a decrease in total and dissolved soy protein content. The effect of pretreatment on soluble protein (black line) shows that there is an increase in the amount of dissolved protein during pretreatment-1 and gradually decreases significantly during pretreatment-2. While the total protein (blue line) shows that the pretreatment process gradually causes protein denaturation.

Figure 1B shows the effect of pretreatment temperature on the amount of dissolved protein in soybean raw material. The amount of dissolved protein increases linearly with increasing temperature. However, the use of higher temperatures causes a decrease in dissolved protein content. This can also be seen in Fig. 1A, where in the process of steaming (pretreatment-2) there is a significant decrease in dissolved protein content. So the use of pretreatment temperature becomes an important parameter to get good raw materials in making *tempeh*.

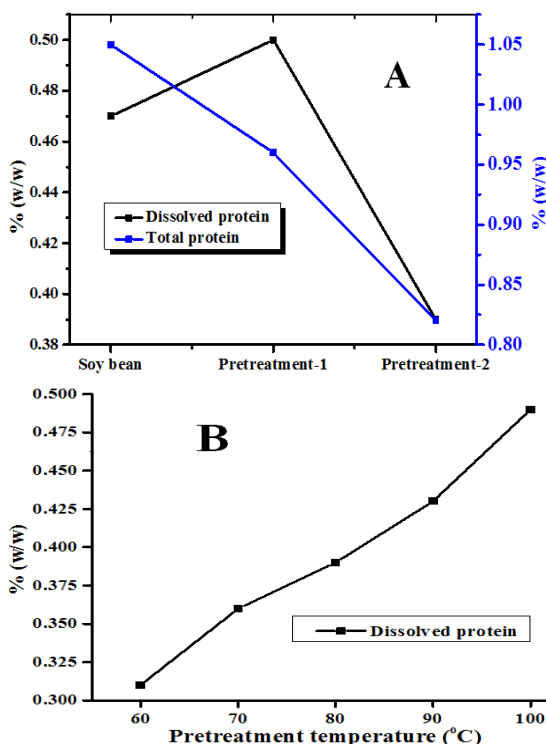


Fig. 1. (A) Effect of pretreatment on protein content; (B) Effect of temperature pretreatment on dissolved proteins

Effect of Solid-State Fermentation (SSF) on *tempeh* protein

Figure 2 shows the effect of SSF time on the protein content of both protein total (blue line) and dissolved protein (black line). The long SSF time causes the total protein content in *tempeh* to increase while the dissolved protein content decreases. Increased total protein associated with the number of mycelia *Rhizopus sp.* which gets more and more as SSF time increases. *Rhizopus sp.* contributed significantly to the total protein content, where the structure of *Rhizopus sp.* which are composed of chitin will contribute to the presence of nitrogen groups. In addition, during the SSF process, *Rhizopus sp.* also produce protein. As for the decrease in dissolved protein content, it can be associated with the activity of *Rhizopus sp.* which denature *tempeh* proteins into amino acids and short chain peptides, and the formation of NH_3 due to *Tempeh* decay.

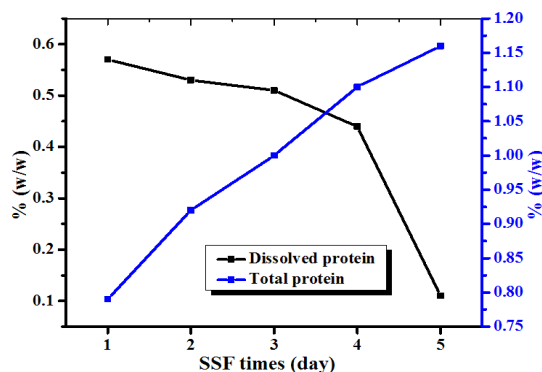


Fig. 2. Effect of SSF times on the content of *Tempeh* protein

Besides the protein content, the time of SSF also affects the acidity (pH) and the water content of *tempeh*. The influence of SSF time on pH is shown in Fig. 3, where the SSF time gradually causes the pH of the *Tempeh* to be higher near neutral pH (pH = 7.0). This characteristic correlates with the amount of denatured protein¹³ and decreased activity of *Rhizopus sp.*^{14,15,16}. The increase in pH is also related to the formation of free amino acids.¹⁷

Figure 4 shows the effect of the SSF time on the *Tempeh* content. Water content decreases linearly with the length of time the SSF. The decrease in water content can be attributed to the reduction in the amount of dissolved protein and damage to the hydrogen bonds either formed between the amide group with water or amide groups with other amide groups. Another factor that causes a decrease in water content is the activity

of microorganisms that damage starch components thereby reducing OH bonds in its structure¹⁸.

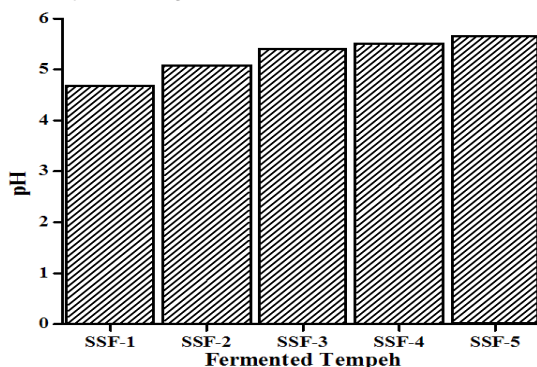


Fig. 3. Effect of SSF time on pH of *Tempeh*

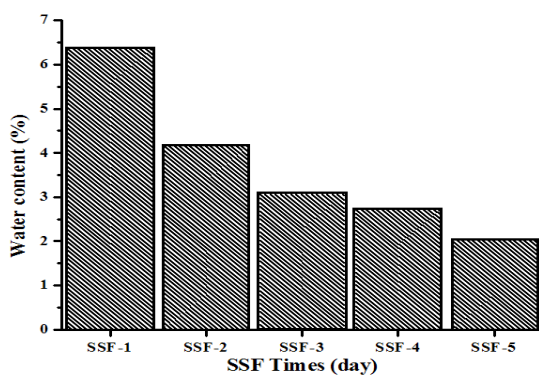


Fig. 4. Effect of SSF time on *Tempeh* water content

FTIR Analysis

Reduction of the amount of *Tempeh* protein both during the pretreatment and fermentation processes was also observed based on the IR uptake of the protein building blocks. Based on its structure, proteins are composed of amino acids linked by amide bonds. This bond consists of groups C = O and N-H with specific IR absorption that occurs at wave numbers in a row that is 1700 cm^{-1} and 1550 cm^{-1} ^{19,20}. Fig. 5 shows the IR spectra of test samples before pretreatment (soybean), after pretreatment, and after fermentation (*Tempeh*). The fundamental difference is seen in the absorption intensity for wave numbers 1700 cm^{-1} and 1550 cm^{-1} . The magnitude of the absorption intensity correlates with the amount of protein contained in the test sample. Soybean

samples (Orange line) show the intensity of specific IR uptake for protein is greater than the intensity of specific IR uptake of samples after pretreatment (Red line) and fermentation (Blue line). This reinforces information about protein denaturation both during the pretreatment process and during fermentation.

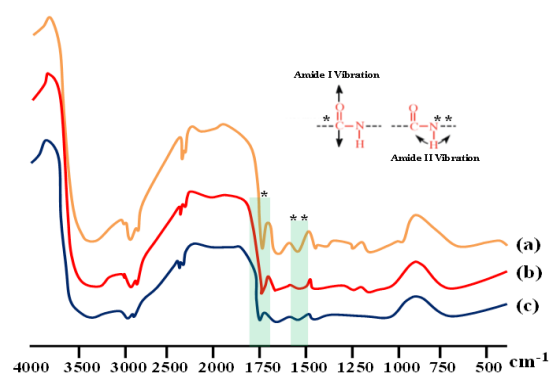


Fig. 5. Specific IR spectra of test samples: a) before pretreatment, b) after pretreatment, c) *Tempeh*

CONCLUSION

The process of making soybean *Tempeh* in general consists of two important stages, namely the pretreatment and solid-state fermentation processes. Based on the results of this study, both processes affect the content of *Tempeh* protein. Where pretreatment temperature and fermentation time are the main factors in decreasing and increasing the protein content. The use of high pretreatment temperatures causes both total protein and dissolved protein to gradually denature. So is the case with the fermentation process.

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Conflict of Interests

We declare that we have no competing financial interests or personal relationships that could affect the work we report in this paper.

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