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Microbiological and Biochemical Tests on Tempe Production Using Tempe Mold Innovation

Siti Harnina Bintari*, Dhimas Fajar Eka Purnama, Danang Dwi Saputro, Sunyoto Sunyoto, Pramesti Dewi, Ibnul Mubarok

Department of Biology, Faculty of Mathematics and Natural Sciences, Univeristas Negeri Semarang, Kampus Sekaran, Gunungpati, Semarang, Indonesia 50229

*Corresponding Author: harnina@mail.unnes.ac.id

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Abstract. Tempe is a popular fermented food in Indonesia, one of the important things in the process of making tempeh is the tempe incubation container. The purpose of this study was to prove the quality of tempe products made using the tempe mold innovation suitable for production, to analyze the comparison between the quality of tempe products made using a tempe incubation tool and without using the tool. Experimental research with Completely Randomized Design (CRD), applying a tempe incubator and without using a tool as a control. Test methods used include testing water content, hygiene, number of mold colonies and protein content. Based on the results of the study, tempe products made using a tempe printer contain a moisture content of 51.4 - 56.2%, protein content 17.92 - 18.58%, the number of mold colonies 3.65 x 10^5 untill 4.08 x 10^5 cell/gr and negative *Escherichia coli*. Based on the results of the study, it was concluded that the tempe products made using the tempe mold innovation tool had met the biological and chemical quality standards based on SNI 3144:2015 and had an overall quality test result that was superior to the control.

Key words: tempe; biochemical tests; innovative incubator; tempe quality.

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INTRODUCTION

Tempe is a popular fermented food in Indonesia, the high consumption of tempeh has led to various innovations in increasing its production, one of these efforts is the simple patent innovation of the tempe incubator. This tool acts as a mold as well as a place for ripening tempeh so that it is able to pour a number of cooked soybeans into the packaging. In addition, the tool is claimed to be able to support the growth of mold cells during the fermentation process. This is due to the aeration of the upper and lower plates of the incubator.

Innovations in food packaging systems will help meet the needs of an ever-evolving market, such as consumer demand for "healthy" and high-quality food products and the reduction of the negative environmental impact of food packaging. So far, tempeh is packaged using one-by-one packaging method, therefore the emerging concept of active and intelligent packaging technology provides many innovative solutions to extend shelf life and improve the quality and safety of food products. The innovative, intelligent and environmentally friendly tempe packaging technology produces a multipurpose food packaging system without negative interactions between components, and this goal can be seen in the tempe incubator which is made for effective tempe packaging. This article informs the packaging of food tempeh and practical, innovative developments and guarantees aeration for the growth of tempeh thread fungus. Global patents and future research trends are also discussed.

The value of hygiene and quality of the resulting product also observe to the basic needs for mold life. Measurement of the strength of tempe is appropriate for quality assessment purposes, but it provides little information about the amount of fungal biomass formed. The food industry is being challenged to develop new healthy food products (Vital *et al.,* 2018). Tempeh, originating from Indonesia and produced by fermenting mushrooms, will be a healthy alternative food for Indonesians. This study was designed to produce tempeh made with an innovative incubator, to determine and compare the biochemical and sensory properties of making tempeh with conventional ones. For engineering studies and fermentation monitoring, the real-time measurement of biomass accumulation is

desirable and the microbial community structure in tempeh is a very important feature in maintaining not only the sensory appearance but also the functional nature of the tempeh. Meanwhile, super critical carbon dioxide technology can be an alternative process for making tempeh which is expected to reduce the number of bacteria and at the same time maintain high mold growth. The high number of bacteria in tempeh can interfere with the growth of fungi and as a result tempe will be damaged more quickly

Tempe production process needs to observe to the presence of oxygen for growth factors, avoiding contamination and sanitation as much as possible in the fermentation process. Almost all stages in the tempeh-making process are critical stages in determining the quality of tempe. According to Bintari (2013), the hygienic and modern process of making tempeh is carried out using two heating techniques, namely heating before and after soaking. Heating after immersion is carried out by means of pasteurization with the aim of killing pathogenic microbes, eliminating anti-nutritional compounds and preventing protein denaturation. According Nugraeni *et al.,* (2016), the common pathogenic bacteria that contaminate food products is *Escherichia coli.* It has a habitat, especially in water sources and contaminants from producer.

According to Jubaidah *et al.,* (2017) Tempe that has good quality must meet the quality of physical and chemical requirements which can be seen from the appearance of the tempeh shape and nutritional content. Tempe quality standards are regulated in SNI 3144: 2015 which includes nutritional content, physical condition, contamination microbes and chemicals. Physical quality characteristics of tempe can be seen from the white color of tempe and have a compact texture, while the chemical quality characteristics can be seen from the nutritional content contained in tempe. Tempe contains various nutrients needed by the body, including 15% protein, 5% fat, 4% carbohydrates, vitamin B12 and is rich in minerals and fiber (Judith *et al.*, 2018).

There are several factors that affect the quality of tempe related to tempe incubation equipment during the fermentation process such as airation, oxygen adequacy, temperature, pH, raw materials and duration of the fermentation process and packaging (Hasrudin dan Pratiwi, 2015). According Sayuti (2015) to produce good quality tempeh products, it is necessary to use a wrapper or curing place that is able to maintain aeration and high humidity conditions without causing condensation. Tempeh ripening temperature is good in the range of 27º-35ºC with fermentation time ranging from 36-48 hours and a good humidity level for mold growth is in the range of 60-80% RH (Sari *et al.*, 2021). Meanwhile, in the research of Salim and Rahayu (2017) fermented tempe products using various types of packaging have different protein levels. This is supported by research from Liuspiani *et al.,* (2020) confirming that various types of packaging used affect the organoleptic quality of tempeh.

So far, tempeh is made using plastic packaging, leaves and paper wrapped manually one by one by producer. This in general will have an impact on the length of time for making tempe. Making tempe from start to finish takes 4 x 24 hours, where for the incubation time it takes 2 x 24 hours. The time allotted to make tempe before the fermentation process is mostly used at the soaking and packaging stages. Packing that is done manually one by one requires a lot of time and a lot of energy. Therefore, the tempe incubator as well as the container/packaging is expected to be a solution for the packaging stage easily and quickly.

The ripening site is one of the important factors in determining the final quality of a tempeh product, so there is a need for further feasibility testing in the use of simple patents for tempe-printing innovations for biological, chemical and organoleptic hygiene and quality in tempe products made using these tools. Quality and hygiene tests were carried out using tempeh which was made without using a mold as a comparison or control. And innovations in food packaging systems will help meet the evolving needs of the market, such as consumer preference for "healthy" and high-quality food products and reduction of the negative environmental impacts of food packaging (Han et al., 2018)

The aim of the research is to prove the biological and chemical qualities of tempeh made with an innovative printer. Analyzing the comparison between the quality of tempeh products made using innovative printers and those that are not. The benefit of the research is to provide information about various tempe incubator packaging tools, which are named innovative incubator.

METHODS

Sample preparation

The procedure for making tempe uses the modern and hygienic method of making tempe which is described in Bintari (2013). Soybean

seeds are washed and boiled for 20 minutes, the epidermis is peeled and soaked for one night then boiled again for 10 minutes using the pasteurization method, the soybean seeds are drained and allowed to cool and dry, the soybeans are then sprinkled with yeast or microbial inoculum evenly, which then treated with packaging techniques which included the use of an incubator with leaf, plastic and paper packaging and without the use of an innovative incubation device as a control (tempe packaging leaves, plastic and paper) and fermented tempeh for 48 hours (Figure.1 and Figure 2).

Microbiological Test

Microbiological test analysis was carried out using PDA medium which was poured into a petri dish with a volume of 25 mL added with anti-bacterial chloramphenicol in a ratio of 1gr/100ml and then incubated for 3 x 24 hours; then macroscopic observations were made using a coloni counter which was then observed for filamentous colonies as a group. cells in a colony are assumed to originate from a single cell. The results of the analysis in the form of numbers are considered to be included in the statistical data to be tested. After observation tempe for 4 days. The data was processed using SPSS 25.

Testing of *Escherichia coli* bacteria on tempeh was carried out using serial dilutions with EMBA medium. The EMBA is weighed at 37.5 grams/liter. In making serial dilutions, 1 gram of fresh tempeh was weighed into 9 ml of sterile distilled water and homogenized using a vortex until the dilution was 10^{-5}. Followed by the inoculation of 10^{-5} suspension samples into the EMBA in a petridish made in duplicate for 2x24 hours. The calculation of *Escherichia coli* bacteria is carried out directly using a colony counter.

Observation of the number of thread fungi on PDA medium in petridish was counted using a coloni counter and counted the number of cells directly in the form of filamentous.

Chemical Test

Water content test

The water content test was carried out using the thermogravimetric method by drying using an oven at a high temperature and weighing until a constant weight was obtained. Determination of the water content begins with weighing the porcelain dish and sample, the sample is put into a porcelain dish and then baked at a temperature of 1050C until a constant weight is obtained (Banobe et al., 2015).

Protein measurement

Analysis of protein content was carried out by the biuret method with steps including making reagents, determining the maximum wavelength, determining the standard curve and testing protein samples. Determination of the maximum

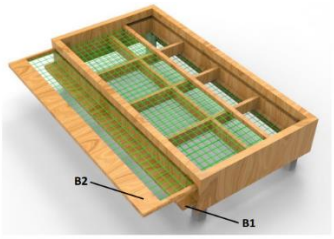

Figure 1. Tempe Mold Inovation

Figure 2. Tempeh with three types of packaging. using innovative incubators with leaf packaging (a); using innovative incubators with using innovative incubators with paper packaging (b); using innovative incubators with plastic packaging (c); leaf packaging control (d); plastic packaging control (e); paper packaging control (f)

wavelength was carried out by measuring the maximum absorption of 3% Bovin Serrum Albumin (BSA) at a wavelength of 400-800 nm (Behkami *et al*., 2019). The standard curve was made using the absorbance blank and BSA solution with concentrations of 1%,2%,3%,4% and 5% at the maximum wavelength, which was then continued with sample testing by measuring absorbance. The protein content in the sample was obtained based on the results of the linear regression equation from the albumin standard calibration curve.

Data analysis

Qualitative data which includes macroscopic observations on tempeh fermentation and E.coli contamination were analyzed descriptively and the results of the data obtained from measurements which included the number of mold colonies, water content and protein content were analyzed using the two way ANOVA test with a significant level of 95%, if there was an effect followed by Duncan's test (Kurniawan *et al.,* 2019).

RESULTS AND DISCUSSION

Macroscopic observations during fermentation

Observations made during the tempeh fermentation process included the growth of mold and the compactness of soybean seeds on tempeh from 0 to 48 hours of fermentation. The results of visual Observation early fermentation until 48 hours fermentations can be seen in (Figure.3).

Overall treatment and control at 0 hour observation showed the same results, namely the absence of growth in the mold and soybean seeds separated from each other. At the 24 hour observation, it was seen that there was mold growth activity that occurred in all of the treatment and control samples, at 24 hours the entire sample showed the mold growth was evenly distributed on

Figure 3. Observation early fermentation until 48 hours fermentations

the soybean surface with small water spots with an opaque color. According Sine dan Soetarto (2020) the spots or water vapor formed in tempe fermentation is the result of the breakdown of carbohydrates carried out by microbes, during the tempe fermentation process, microbes will digest the substrate and produce water, carbon dioxide and a number of ATP as products. During the tempeh fermentation process, microorganisms digest the substrate and produce water, carbon dioxide and large amounts of energy (Sine *et al*., 2020). Meanwhile, the observation of compactness in soybean seeds showed that the overall sample was not compact, soybean seeds were separated from each other, this was because the growth of *Rhizopus oligosporus* at 24 hours had not reached the optimal stage so that the mycelium could not bind perfectly between soybean seeds to each other. At 12 to 24 hours tempeh fermentation is an acceleration phase, at this stage the cells of the mold begin to divide and enter the active phase (Wahyudi, 2018).

Visual observation of mold growth at 48 hours on samples of leaf, plastic and paper coating mold treatment as well as leaf control, plastic and paper obtained results that *Rhizopus oligosporus* grew evenly covering the entire surface of tempe with dense mycelium growth penetrating soybean seeds. Meanwhile, for the overall compactness of the samples, both treatment and control showed compact results with very tight mycelium so that the soybean seeds condensed to have an appearance like tempeh. These results indicate that all samples exhibit the ideal characteristics of tempeh, which have evenly distributed white mycelium with a compact appearance (Kustyawati *et al*., 2018).

Biological quality

Escherichia. coli contamination

Tests for suspected E. coli contamination were carried out using EMBA selective medium. EMBA contains eosin and methylene blue which will inhibit the growth of gram-positive bacteria, besides the lactose carbohydrates contained in this medium will cause the gram-negative bacteria to differentiate.

When the sample is positive for E. coli it will be marked by a green color due to the deposition of methylene blue as a result of increasing acid levels due to lactose fermentation (Kim *et al*., 2016). The test results of suspected *Escherichia coli* contamination are presented in (Table 1).

The results of the *E. coli* bacterial contamination test showed that the entire sample was negative, so it can be concluded that the tempe product made using an innovative tool for making tempeh with various types of coatings has met the quality requirements of soybean tempeh (SNI 3144:2015), which is a maximum contamination of 10 cells/gram. The negative results of the entire sample were due to the possibility of very few *Echerichia. coli* bacteria contained in the sample.

Tabel 1. Table of *Escherichia coli* contamination

Sample	The Result of Alleged *E. coli* contamination (+ / -)		
	Reduplication		
	1	2	3
Leaf control	(-)	(-)	(-)
Leaf coating tool	(-)	(-)	(-)
Plastic control	(-)	(-)	(-)
Plastic coating tool	(-)	(-)	(-)
Paper control	(-)	(-)	(-)
Paper coating tool	(-)	(-)	(-)

The application of a modern and hygienic method of making tempeh by means of 2 times heating based on Bintari (2013), has a major role in determining the negative results for *E. coli.* The process of heating or boiling soybeans in the manufacture of tempeh is able to soften the seeds and deactivate the trypsin inhibitor of soybeans so that they can kill pathogenic microbes and reduce the unpleasant odor of soybeans (Ari and Priambudi, 2020). According to Kusuma and Dewi (2016) heating at a temperature of 70ºC and above, pasteurization and chlorination from 0.5 to 1 ppm can kill coliform bacteria in food.

During the soaking process, soybeans will decrease until it reaches 5.3 – 4.5 due to lactic acid bacteria. Lactic acid bacteria can produce antibacterial compounds in the form of organic acids, hydrogen peroxide, diacetyl, acetaldehyde and bacteriocins which are antibacterial compounds against several types of pathogenic bacteria such as *Staphylococcus aureus* and *Escherichia coli* (Suardana *et al.,* 2016). In addition, the relatively low moisture content of the tempeh samples also affected the results. High water content will cause contaminant bacteria to easily grow and develop, thereby accelerating the spoilage of food (Radiati *et al.,* 2016).

Number of Rhizopus oligosporus Cells

The mold colonies formed after incubation for 3 x 24 hours resulted in white to gray colonies with mycelia spreading over the PDA media. *Rhizopus*

oligosporus has the characteristics of a white mycelium with gray spots spreading on the surface of the culture, the color of the colony on the upper side is white with a concentration of thickness in the middle while the edges are thinner (Duniaji *et al.*, 2019). The average results of the calculation of mold colonies can be seen in (Table 2).

Table 2. Table of average number of mold Colonies

Treatment	Average Mold Colony (Colony/gr)
Leaf control	3.78×10^5
Leaf coating tool	4.08×10^5
Plastic control	3.33×10^5
Plastic coating tool	3.65×10^5
Paper control	3.70×10^5
Paper coating tool	3.93×10^5

The results of the calculation of the average number of mold colonies from the treatment using molds ranged in the range of $3.65 \times 10^5 - 4.08 \times 10^5$ while the control showed results in the range $3.33 \times 10^5 - 3.78 \times 10^5$ colonies/gr. Tempe with the treatment using a tempe printer has the highest colony average compared to the control. This is due to the war of air holes contained in the mold tool. If the aeration goes well, the environmental conditions of tempe will be maintained so that the mold will grow optimally (Tahir *et al.*, 2018).

The two-way ANOVA test showed that there were differences in the number of mold colonies from the treatment using molds and packaging variations marked with $p<0.05$, but there was no interaction between the treatment using tempeh molds and packaging variations on the number of mold colonies ($p>0.05$). Duncan's further test results showed that there were significant differences in the treatment of packaging variations from the use of plastic to leaves and paper, but there was no significant difference from the use of leaves and paper. This significant difference is because the leaf and paper type wrappers have even pores which allow better air circulation. A little aeration can inhibit the growth of mold due to lack of oxygen, but if aeration is too much mold will grow quickly and sporulation occurs. Incubation conditions greatly affect the growth of molds and the formation of mycelium, these molds will grow unevenly if aeration is not good, on the contrary if oxygen too much will cause sporulation which results in black spots on tempeh (Laksono *et al.*, 2019). If the aeration goes well, the environmental conditions of tempe will be maintained so that the mold will grow optimally. Aeration is one of the important factors in determining the quality of tempe products (Tahir *et al.*, 2018).

Chemical Quality

Water Content

Food products with relatively high water content are very prone to overgrown with microorganisms that can damage food (Radiati, 2016). Likewise with tempeh, tempeh also has a maximum water content limit in determining its quality. The results of the average water content test can be seen in (Table 3).

Table 3. Table of water content test results

Treatment	Average water content ± sd (%)
Leaf control	55.80 ± 0.07
Leaf coating tool	51.40 ± 0.49
Plastic control	58.85 ± 0.60
Plastic coating tool	56.20 ± 0.78
Paper control	55.25 ± 0.53
Paper coating tool	53.05 ± 0.47

The results of the measurement of the water content of the entire behavior of using tempeh molds were obtained in the range of 51.4 - 56.2% and the control ranged from 55.8 - 58.85%. Based on these results, it shows that tempe products made using a tempe printer with various types of coatings have met the quality requirements of soy tempeh (SNI 3144:2015) which is a maximum of 65%.

The results of the water content test showed that the overall treatment using the tempe mold tool had a lower water content than the control from each packaging variation. This is because the tempe mold is equipped with a cavity from the lattice on the top and bottom surfaces which allows air circulation to run better so that the temperature is stable. In addition, microbial activity and the boiling process in the manufacture of tempeh also affect the water content of tempe. According Lelatobur *et al.* (2016) in tempe fermentation, the fungus digests the substrate and produces water and ATP, but when substrate denaturation occurs the mold will only use the available substrate and produce water and ATP equivalent to the available substrate. The ability of water penetration into the seed matrix on boiling and soaking causes the expansion of soybean seed volume which results in high or low water content in tempe (Laksono *et al.*, 2019). The ability of water penetration into the seed matrix on boiling

and soaking causes the volume of soybean seeds to expand which results in high or low water content in tempe (Syamsuri *et al.,* 2020).

The two-way ANOVA test showed that there were differences in the number of mold colonies from the treatment using molds and packaging variations marked with $p<0.05$, but there was no interaction between the treatment using tempeh molds and packaging variations on the number of mold colonies ($p>0.05$). Based on the results of Duncan's further test, it showed that there were significant differences from all the various packaging treatments, both from the use of leaves, plastic and paper. This significant difference is caused by the permeability of each package, according to Jayadi *et al*. (2018) plastic packaging has a lower permeability to water vapor and gas than leaves, so plastic packaging is good at maintaining the state of food ingredients from weight loss due to evaporation of water content.

Protein content

Measurement of Protein Levels in Tempe

The data on the determination of the protein content of the tempeh samples made using an innovative tempe printer and their respective controls can be seen in (Table 4).

Tabel 4. Result of determination on protein content

Treatment	Protein content (%
Leaf control	17.51
Leaf coating tool	18.58
Plastic control	16.83
Plastic coating tool	17.92
Paper control	17.21
Paper coating tool	18.42

This data shows that the average protein content of tempe products made using a tempe printer has a range of 17.92 – 18.58%. As for the control ranged from 16.83 - 17.51%. These results indicate that tempeh products made using an innovative tempe printer with various types of coatings have met the quality criteria of soybean tempeh based on SNI 3144:2015, which is at least 16%.

The results of the two-way ANOVA test of protein content of tempe products made using an innovative printer indicated that there were differences in the protein content of tempe products made using innovative printers with various packages marked with $p<0.05$. The results of Duncan's test showed that there was a significant difference in the treatment of variations in plastic packaging on paper and leaves, but there was no significant difference in the treatment of variations in paper packaging with leaves. According Winarti dan Wicaksono (2020), The type of packaging affects the protein content of tempeh. Leaf and paper packaging that has evenly distributed pores is good for maintaining air circulation, resulting in optimal mold growth. Protein content in tempeh is strongly influenced by the proteolytic activity of *Rhizopus oligosporus. Rhizopus oligosporus* is a protease enzyme-producing mold that functions to break down protein complex nutritional compounds Rhizopus oligosporus is a protease enzyme-producsing mold that functions to break down protein complex nutritional compounds in the form of peptides and free amino acids (Nurholipad and Ayum, 2021). During fermentation, protease enzymes will be produced in large quantities and will break down amino acids and other solutes so that they are easily absorbed by the body (Astawan *el al.,* 2013; Sayuti, 2015). Hydrolysis of protein is only used about 5% to become a source of carbon and energy, the rest will accumulate into the form of peptides and amino acids. The protease activity in fermenting soybeans into tempeh only requires 5% of the protein which is hydrolyzed into a source of carbon and energy, the rest of the degradation during the process will accumulate into the form of amino acids and peptides in tempe products (Mahadi, 2016). Optimal mold growth resulted in the optimal work of the protease enzyme in degrading protein into free amino acids so that the dissolved protein would be higher.

Empirically, tempeh printing can be done manually by hand in leaf, plastic or paper packaging. Then, with the increase in the number of packages made for each manufacture of tempe, it is necessary to use an inexpensive method and a precise weight of tempe. The innovative tempe incubator provides an alternative to both packaging and printing tempeh which can be wrapped in leaves or paper or plastic. The benefits of this research are as an alternative to printing and packaging tempeh at the same time.

CONCLUSION

From all the tests that have been carried out, the use of an innovative incubator and the type of packaging have an effect on protein content, moisture content and the number of mold colonies, the number of mold colonies but has no effect on the level of hygiene of tempe, besides tempe products made using innovative molds have met the quality standards of biological and chemical

quality based on SNI 3144:2015 and have overall quality test results that are superior to the control.

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