Jurnal Riset Biologi dan Aplikasinya, Volume 4, Issue 1, March 2022

Jurnal Riset Biologi dan Aplikasinya
https://journal.unesa.ac.id/index.php/risetbiologi

Antibacterial Activity of Garlic Added to Tempeh against *Bacillus* sp. and *Escherichia coli*

Adelia Ayu Fannya Prasetya, Lusiawati Dewi*, Risya Pramana Situmorang
Faculty of Biology, Satya Wacana Christian University, Jl. Diponegoro 52-60 Salatiga - Indonesia
*Corresponding Author
e-mail: lusidewi804@gmail.com

Article History

Received : 24 February 2022
Revised : 19 March 2022
Approved : 21 March 2022
Published : 31 March 2022

Keywords
antibacterial activity, fermentation, garlic, tempeh

ABSTRACT

Tempeh (*tempe*) has been known to have antibacterial properties due to the presence of glycoprotein compounds, antimicrobial peptides, and flavonoids produced by the fungus *Rhizopus* spp. during the fermentation process. The addition of garlic powder is expected to increase the antibacterial activity of tempeh against contaminant bacteria. This research aimed to analyze the antibacterial activity, water content, and organoleptic properties (taste, aroma, and color) of tempeh with garlic powder supplementation. Garlic powder was added to the tempeh fermentation process with various concentrations of 0.15, 0.25, 0.50, and 0.75%. Antibacterial activity, water content, and qualitative organoleptic tests were analyzed using paper disk diffusion, gravimetric, and questionnaire methods. The results showed that the addition of garlic powder in all treatments for *Bacillus* sp had shown an inhibitory activity with moderate strength with an inhibitory power of 0.15; 0.25; 0.50; and 0.75% respectively 8±1.71; 10±1.71; 7±0.96; 9±0.58. Meanwhile, *Escherichia coli* showed no inhibitory activity. The addition of garlic powder at a concentration of 0.25% reduced the water content to 58.25±0.03. In addition, the addition of garlic powder was not able to affect the color but was able to influence the aroma and taste of *tempe*h. Garlic powder was able to inhibit *Bacillus* sp with the best concentration of 0.25%; the addition of garlic powder can reduce the water content and affect the aroma and taste of tempeh.

How to cite: Prasetya, A.A.F., Dewi, L & Situmorang, R.P. (2022) Antibacterial Activity of Garlic Added to Tempeh against *Bacillus* sp. and *Escherichia coli*. *Jurnal Riset Biologi dan Aplikasinya*, 4(1):34-41. DOI: 10.26740/jrba. v4n1.p34-41.

INTRODUCTION

Tempeh (*tempe*) is a fermented food that is highly demanded among Indonesia's due to its high protein. According to Banobe *et al.* (2019), Tempeh made from soybeans has a protein content of 27.42%, fat content of 15.94%, carbohydrate content of 49.38%, and water content of 5.43%. A fermentation process using *Rhizopus* spp. is required to make tempeh. The fermentation process will result the formation of white hyphae that resemble thread strands that will envelop soybeans. The number of hyphae will be affected by the duration of the fermentation, and if there are more, they can bind soybeans into a single unit. Because of the dense mycelium, the tempeh structure will become compact and dense (Barus *et al.*, 2019).

During the fermentation process, various types of natural additives can be added to tempeh, such as spices to improve the product's nutrition, taste, function, and attractiveness. According to Rahmi *et al.* (2018), garlic (*Allium sativum*) is one of the sources of natural additive. Allicin, alliinase, S-allil cysteine, diallyl sulfide, and allyl methyl trisulfide are all found in garlic bulbs. When compared to other types of thiosulfate compounds, Allicin have the highest composition, approximately 70-80%. This allicin compound is responsible for the flavor and aroma of garlic. The allinase enzyme converts the aline compound into Allicin when the garlic

bulb is destroyed (Moulia *et al*., 2018). In addition, Allicin has antimicrobial properties for both gram-positive and gram-negative bacteria, as well as antifungal properties (Salima, 2015).

Basically, *Rhizopus* spp. can produce antibacterial compounds such as glycoproteins, antimicrobial peptides, and flavonoids on their own during the tempeh fermentation process (Noviana *et al*., 2018). The addition of garlic powder is expected to be a solution for increasing the inhibitory power on the growth of unwanted gram-negative and gram-positive bacteria. The addition of garlic to the tempeh fermentation process can achieve the proper concentration of allicin compounds to inhibit contaminant bacteria in tempeh. However, another potential finding is that the identification process of excessive concentrations has the potential to inhibit the metabolic process and growth of *Rhizopus* spp. This antibacterial property can be tested by looking at a bright inhibition zone around the colony's growth. Because the active compounds in the extract inhibit the bacterial growth, this zone indicates the absence of it (Mawaddah *et al*., 2018).

In this study, garlic bulbs was powdered and added to the tempeh fermentation process at various concentrations to increase the nutritional value and antibacterial properties tempeh. Through this process, allicin compounds from garlic bulbs that are antibacterial are not removed to determine the level of antibacterial activity or strength produced by adding garlic bulbs during the tempeh fermentation process. Rahmi *et al.* (2018) experimented with the addition of garlic during the tempeh fermentation process, and it has been shown to improve the quality of tempeh's taste, aroma, color, and texture. But the difference is the research conducted by (Rahmi et al., 2018) only reviewed the nutritional content, even though the compounds contained in garlic have the potential to not only increase nutritional content but also have the potential to inhibit contaminant bacteria if tempeh is made in an unhygienic way.

According to Rahmi *et al.* (2018), garlic can also increase protein levels in tempeh. However, it can also increase several types of essential and non-essential amino acids, even though not significantly. It might because of the soybeans was soaked in garlic and steamed for 15-20 minutes. This treatment causes the loss of allicin compounds attached to soybean seeds since these compounds are thermolabile. Allicin compounds have numerous health benefits, including being a source of antioxidants that can prevent the body producing nitrate dioxide (Sudjatini, 2020), having antidiabetic properties (Lisiswanti and Haryanto, 2017), and being able to lower blood pressure, reduce platelet aggregation, prevent hyperlipidemia and boost immunity (Rahman, 2014). This study aimed to determine the effect of garlic powder addition to the antibacterial activity, water content, and organoleptic properties of tempeh.

MATERIALS AND METHODS

This research was conducted in September – November 2021 at the Microbiology and Biochemistry Laboratory, Faculty of Biology, Satya Wacana Christian University. The materials used in this study included garlic bulbs (*Allium sativum)*, boiled soybeans from tempeh maker in Gamasan, Bandungan, Central Java, tempeh yeast (RAPRIMA, Indonesia), ethyl acetate (Degrees of Analysis, Merck Millipore, Germany), nutrient agar (Merck Millipore, Germany), NaCl (Degrees of Analysis, Merck Millipore, Germany), tetracycline antibiotics 30 mcg (Oxoid, USA), filter paper, and disk paper (Oxoid, USA).

Samples preparation

Garlic powder was made by drying garlic bulbs in a dehydrator at 60°C for 1–3 hours. When the garlic bulbs have dried, then crushed using blender until turned into powder and ready to be added in tempeh fermentation process. The process of making tempeh begins by inoculating 2 grams of starter inoculum into 100 grams of boiled soybeans, then garlic powder was added with concentrations of 0%(control), 0.15, 0.25, 0.50, and 0.75%. The mixtures were put in a plastic ziplock and then punched with a toothpick to keep oxygen circulating during the tempeh fermentation. Then incubated at room temperature for 48 hours.

Antibacterial activity assay

An antibacterial test was carried out using a disk diffusion assay. The tempeh was extracted by maceration method using ethyl acetate. Fifty grams of mashed tempeh were mixed with ethyl acetate until all sample parts were submerged. The container was closed and stored at room temperature in the dark for three days under stirring conditions at a speed of 150 rpm. The ethyl acetate solvent was changed daily to maximize the obtained extract. The macerate was concentrated using a rotary vacuum evaporator at 45°C. The resulting thick section was aerated with nitrogen gas to evaporate the remaining solvent completely. The antibacterial test was carried out against

Bacillus subtilis ATCC 6275 and *Escherichia coli ATCC 8739*. The 0.5 MFU bacteria were prepared and spread on NA media. The paper disk containing the extract under various concentrations was placed in the plate. Tetracycline was used as a positive control. The samples were incubated at 37°C for 24 hours. Antibacterial activity was seen by measuring the diameter of the inhibition zone formed around the colony. The strength category of antibacterial activity was based on (Mawaddah et al., 2018) namely ≤ 5 mm, weak; 5–10 mm, medium; 10–20 mm, strong; ≥ 20 mm, very strong. To determine the antibacterial group, the media was incubated for 5 days at 37°C to determine whether the inhibition zone remains or disappears. The results then tested statistically using one way anova.

Water content assay

The water content test was carried out by the gravimetric method. The porcelain cup was heated using an oven at 100°C for 1 hour and cooled using a desiccator for 20 minutes. The weight of the cup was obtained by measuring the cold porcelain cup (W0). The tempeh samples were weighed as much as 2 grams using an analytical balance. Then the samples were placed into a porcelain cup. Before being heated, the porcelain cup and samples were weighed using an analytical balance (W1) and then heated using an oven at 100°C for 5 hours. Furthermore, the porcelain cup was cooled using a desiccator for 20 minutes and weighed using an analytical balance. The heating process was repeated until a constant weight (W2). The water content in tempeh was calculated using the formula (Laksono *et al*., 2019):

$$\text{Water Content (\%)} = \frac{W1-W2}{W1-W0} \times 100\%$$

The results then tested statistically using one way anova.

Organoleptic test

In the organoleptic test, the color, aroma, and taste were observed. This test was conducted on 25 untrained panelists. Tempeh is fried with the same standard of fire and old. The scores for the observed indicators are: 5=very preferable, 4= preferable, 3= neutral, 2= dislike, 1= very dislike. The results then tested statistically using Friedman test.

RESULTS AND DISCUSSION

The effect of garlic powder addition to the antibacterial activity of tempeh

Antibacterial activity is a test used to determine the strength or ability of antibacterial substances to inhibit the growth of bacteria (Afriani et al., 2017). The results of the One Way Anova test with a 5% level of antibacterial activity against *Bacillus sp* showed that each treatment with the addition of garlic powder showed a significant difference in the effect on the antibacterial activity of tempeh ($p<0.05$) (Table 1). The result shown inhibition of the growth of *Bacillus sp.* that might be caused by an antibacterial compound from Allicin (Table 2, Figure 1). This compound has been known to have antimicrobial activity by blocking the synthesis of RNA, DNA, and protein (Purwantiningsih *et al*., 2019). Allicin compounds can also inhibit the synthesis of phospholipids in the bacterial cell wall, which results in increases the permeability of the cell wall (Purwantiningsih et al., 2019). Increases the permeability of cell wall also causes the sulfhydryl and disulfide groups in cystine and cysteine to undergo lysis, as a result protease enzymes cannot synthesize and the cell proliferation process becomes inhibited (Purwantiningsih *et al*., 2019).

In addition, garlic contains several flavonoid compounds that can interfere with cell membrane permeability by forming hydrogen bonds with extracellular proteins and integral proteins on bacterial cell membranes. This bond involves absorption reactions and occurs in the hydrophilic part of the membrane. Furthermore, the phenol–protein complex will enter the cell membrane and cause protein precipitation, resulting in the inactivation of enzymes in bacteria, disruption of the nutrient transport process, and increased membrane permeability (Purwantiningsih *et al*., 2019). Increased membrane permeability can also cause metabolic enzymes to diffuse out of the cell so that the reaction for the formation of ATP as an energy source for cell growth cannot run properly (Mawaddah *et al*., 2018). Based on research conducted by Soraya *et al.* (2018), garlic also contains tannin compounds that can inhibit the performance of proteolytic enzymes so that protein macromolecules cannot be broken down into their constituent amino acids. As a result, bacteria cannot use protein molecules to support their growth.

The bacterial inhibition mechanism can also be caused by the presence of glycoprotein compounds,

Table 1. One way anova test of antibacterial activity of garlic tempeh against *Bacillus* sp.

Anova					
	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	19.800	4	4.950	3.375	.037
Within Groups	22.000	15	1.467		
Total	41.800	19			

Table 2. Strength category of antibacterial activity of garlic tempeh against *Bacillus* sp.

Treatments	**Antibacterial Activity**	
	Inhibition Zone Diameter (mm)	**Category***
Positive control (Tetracycline)	25	Very strong
Negative control (0 %)	8±0.50	Medium
0.15%	8±1.71	Medium
0.25%	10±1.71	Medium
0.50%	7±0.96	Medium
0.75%	9±0.58	Medium

Note: *Category based on (Mawaddah *et al*., 2018) ≤ 5 mm, weak; 5–10 mm, medium; 10–20 mm, strong; ≥ 20 mm, very strong

Table 3. Strength category of antibacterial activity of garlic tempeh against *Escherichia coli*

Treatments	**Antibacterial Activity**	
	Inhibition Zone Diameter(mm)	**Category***
Positive control (Tetracycline)	27	Very strong
Negative control (0%)	0±0	No inhibitory zone
0.15%	0±0	No inhibitory zone
0.25%	0±0	No inhibitory zone
0.50%	0±0	No inhibitory zone
0.75%	0±0	No inhibitory zone

Note: *Category based on (Mawaddah *et al*., 2018) ≤ 5 mm, weak; 5–10 mm, medium; 10–20 mm, strong; ≥ 20 mm, very strong

antimicrobial peptides, and flavonoids produced by the fungus *Rhizopus spp.* itself (Noviana *et al*., 2018). Based on Noviana *et al.* (2018), glycoprotein compounds can bind to iron which acts as a cofactor for various enzyme performances in the cellular respiration system and as a cytochrome component in bacterial cells, the presence of this bond will cause iron deficiency and inhibit the growth *Bacillus* sp. Glycoprotein compounds can only inhibit the growth of *Bacillus sp.* as a gram-positive bacteria because its cell structure is simple and it has a thin cell wall ranging from 25-30 µm. The cell wall consists of one layer composed of 90% peptidoglycan and 1–4% lipids, so it causes glycoproteins can penetrate cell walls more easily (Noviana *et al*., 2018). Then antimicrobial peptides are also able to act as antibacterial compounds by modifying the outer structure of the cell membrane, which causes depolarization and increased membrane permeability, that these peptides are also able to inhibit the synthesis of proteins, DNA, and RNA from bacteria (Noviana *et al*., 2018). Furthermore, flavonoid compounds act as antibacterial agents by disrupting cell membrane permeability (Noviana *et al*., 2018). The presence of natural antimicrobial compounds in tempeh caused the control treatment to show inhibitory activity against *Bacillus* sp.

The inhibition category was classified as moderate because the diameter of the inhibition zone against *Bacillus* sp. was about 5-10 mm (Table 2). It might be caused by the concentration of garlic powder being too small so that the effect on

antibacterial properties was not significant. Furthermore, Allicin has unstable properties during storage at room temperature; therefore, the compound will be further metabolized into an ajoene compound, which has another name, diallyl disulfide or vinylidene (Purwantiningsih et al., 2019). The presence of ajoene compounds causes the low antibacterial properties of garlic tempeh because this compound has a antibacterial properties weaker than allicin (Purwantiningsih *et al*., 2019). Another factor might be caused by the extraction process, which cannot completely extract polar compounds. The solvent used in this study was ethyl acetate, which has semi-polar properties so that compounds such as flavonoids cannot be adequately extracted (Mawaddah *et al*., 2018). Based on research conducted by Kemit *et al.* (2016), flavonoids are polar compounds due to their structure being composed of hydroxyl groups, so this means only solvents that have polar properties are able to extract them completely.

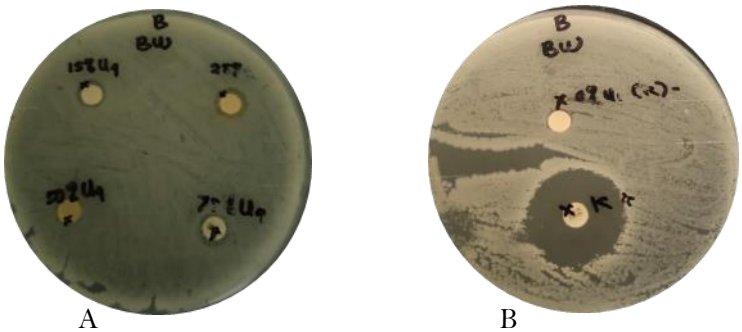
The antibacterial compounds in tempeh have a bacteriostatic mechanism of action (Figure 3). That can only inhibit the growth of *Bacillus* sp. bacteria without killing it because when incubated for 24 hours at 37°C, it can show inhibitory activity, but when further incubated for up to 5 days. The clear zone, which indicates the inhibition zone, has begun to disappear (Mawaddah *et al*., 2018). Based on the strength test of antibacterial activity, the results showed that the addition of garlic powder at a concentration of 0.25% showed the best inhibitory power against *Bacillus* sp. bacteria. In contrast, the higher concentration of garlic powder showed decreased antibacterial activity. It might be due to the garlic powder inhibiting the growth of *Rhizopus* spp., hence natural antibacterial compounds in tempeh cannot be produced properly (Noviana *et al*., 2018).

Although the garlic tempeh extract showed antibacterial activity against *Bacillus* sp. (Table 3, Figure 2), its active compounds could not inhibit the growth of *E. coli* bacteria because there is no inhibition zone formed. It might be due to *E. coli* belonging to gram-negative bacteria, which has a more complex cell wall component. It consists of three layers lipoproteins, lipopolysaccharides, and peptidoglycan (Noviana *et al*., 2018).

The lipoprotein layer contains hydrophilic protein porin, making it difficult for antimicrobial compounds to enter bacterial cells (Noviana *et al*., 2018). The antibacterial activity of allicin against gram-negative bacteria occurs through 2 mechanisms, that is damaging the cell wall and inhibiting the synthesis of RNA molecules (Lestari et al., 2018). The process of cell wall destruction occurs by inhibiting the formation of the peptidoglycan layer, which gives strength to the bacterial cell wall. Allicin compound is able to penetrate the lipopolysaccharide layer of gram-negative bacteria and cause membran permeability to increase. Allicin is also able to attack the phospholipid layer and cause it to break down into glycerol and phosphoric acid then followed cell lysis (Lestari et al., 2018). Then the process of of inhibiting RNA synthesis occurs by forming bond with the DNA dependent RNA polymerase enzym (Lestari et al., 2018).

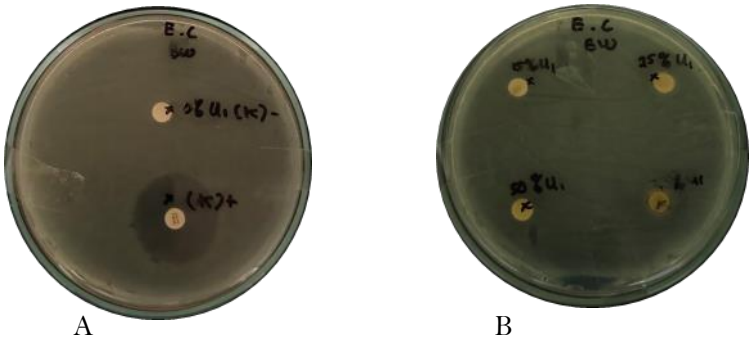
The effect of garlic powder added to the water content of tempeh

Tempeh without garlic powder treatment gets the highest percentage of water content, which was 62.50±0.03% (Table 2). The addition of garlic powder reduces tempeh's water content up to 58.25±0.03% at a concentration of 0.25%. It shows that the concentration of 0.25% garlic powder did not inhibit the growth of the *Rhizopus* spp. The decreasing of water content happen because of the water produced through the fermentation process can still be reused by *Rhizopus* spp. to carry out the respiration (Purwanto and Weliana, 2018). The respiration process often involves the performance of hydrolytic enzymes from Rhizopus spp. so, the decreasing water content indicates the fermentation activity of *Rhizopus* spp increase. This is due to in this hydrolysis process, the free water contained in the substrate will be reduced and used as a reagent and be converted into bound water (Budiono, 2016). This statement was also similar to Purwanto and Weliana (2018), the high rate of respiration and fermentation activity could reduce the percentage of the water content of tempeh. Meanwhile, the addition of garlic powder under concentrations of 0.50 and 0.75% increases the water content tempeh. This could be due to decreased respiration rate due to damage to hydrolytic enzymes from the fungus Rhizopus spp due to thiosulfinate compounds (Allicin), so growth and fermentation activity decreased (Purwanto and Weliana, 2018). But beside on Table 4 all treatments did not show any difference with the control because the significance value was >0.05. Based on the results of all water content measurements obtained, it is possible to conclude that all treatments produced results that met the SNI standard of 65 %.



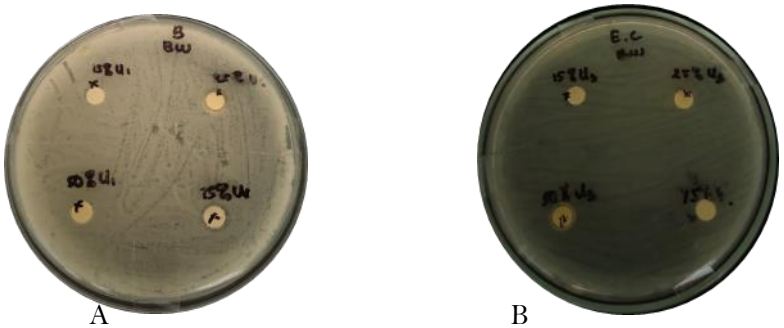
A B

Figure 1. Antibacterial activity of garlic tempeh against *Bacillus* sp bacteria. Note: Negative control and Positive control (A), Treatment of garlic tempeh with concentration of 0.15, 0.25, 0.50, and 0.75% (B)



A B

Figure 2. Antibacterial activity of garlic tempeh against E. coli bacteria. Note: Negative control and Positive control (A), Treatment of garlic tempeh with concentration of 0.15, 0.25, 0.50, and 0.75% (B)



A B

Figure 3. Antibacterial activity after incubation for 5 days. Note: *Bacillus* sp (A), *Escherichia coli* (B)

Table 4. One way anova test of water content on tempeh added with garlic

ANOVA

Water_Content

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	47.300	4	11.825	1.513	,248
Within Groups	117.250	15	7.817		
Total	164.550	19			

Table 5. Value of water content of tempeh added with garlic with different concentrations

Concentration (%)	Water Content (%)*
Control	62.50±0.03
0.15	59.75±0.02
0.25	58.25±0.03
0.50	60.13±0.02
0.75	61.25±0.04

Table 6. Organoleptic test results of garlic tempeh

Parameters	Treatments (Rating 1-5)					Significance Value of Friedman Test
	0%	0.15%	0.25%	0.50%	0.75%	
Color	3.5	3.4	3.4	3.4	3.2	0.685
Aroma	3.2	3.2	3.9	3.0	2.9	0.014
Taste	3.6	3.4	4.0	3.04	3.2	0.025

The effect of the addition of garlic powder to the organoleptic properties of tempeh

The organoleptic test aims to determine the level of presence of the panelists to tempeh added with garlic powder at different concentrations. Table 6 shows the results for the color parameters of all types of tempeh obtained a fairly good value, that met on a scale of 3, and for concentration of 0.15, 0.25, 0.50%, the same value was obtained, that is 3.4. hence it can conclude that all treatments were well received by the panelists. Then for the aroma, the results obtained for concentrations 0f 0.15, 0.25. 0.50% also obtained a good assessment and a concentration 0.25% which has the highest value. And the concentration of 0.75%, the lowest value was obtained because the addition of garlic powder that was too high could cause a pungent odor due to organosulfur compounds (Pratama, 2017). Then for the taste, all types of tempeh can be well received by the panelists and the concentration 0f 0.25% which has the highest value.

Based on the Table 6, it shows that all treatments had no significant effect on color parameters ($p > 0.05$). It is because garlic powder only contained flavonoid, which has colorless characteristics, and flavone that gave yellowish-white color to garlic. According to Zhafira (2018), the addition of garlic powder tends not to contribute significantly to the color of tempeh (Zhafira, 2018). For the following parameter, garlic powder affected the aroma of tempeh ($P<0.05$) because garlic bulbs contained allicin compounds. Although allicin compounds are easily oxidized, the oxidation product still forms organosulfur compounds (Pratama, 2017). In terms of taste parameters, the results of adding garlic powder could affect tempeh's taste. The panelists recommended the highest concentration of 0.25 %. This could be because the application of garlic powder at that concentration does not inhibit the fermentation process by *Rhizopus* spp, so it does not affect the original taste of tempeh. On the other hand, the presence of organosulfur compounds in garlic such as allin, S-methyl-cysteine, allinase, S-propylcysteine, Allicin, S–allyl cysteine, S–ethyl–cysteine, dialill sulfide, allyl methyl trisulfide, dialill disulfide, dialill trisulfide, and methyl allyl trisulfide (Moulia et al., 2018).

CONCLUSION

Based on the findings, the addition of garlic powder to the tempeh fermentation process affected the antibacterial activity for *Bacillus sp.* and the concentration of 0,25%, which provided the most excellent inhibitory activity but had no effect on *E. coli.* Aside from that, the addition of 0.25 percent garlic powder reduced the water content to58.25±0.03. The addition of garlic powder can then affect the aroma and taste of tempeh in the organoleptic test.

REFERENCES

Badan Standardisasi Nasional. 2015. Tempe Kedelai. In Tempe Kedelai SNI 344 : 2015. Badan Standardisasi Nasional, Jakarta.

Afriani, N., Yusmarini, & Pato, U. (2017). Aktivitas Antimikroba *Lactobacillus plantarum 1* yang Diisolasi dari Industri Pengolahan Pati Sagu terhadap Bakteri Patogen *Escherichia coli FNCC-19* dan *Staphylococcus aureus FNCC-15*. *Jom Faperta*, *4*(2), 1–12.

Banobe, C. ., Kusumawati, I. G. A. W., & Wiradnyani, N. . (2019). Nilai Zat Gizi Makro dan Aktivitas Antioksidan Tempe Kedelai (*Glycine maz L.*) Kombinasi Biji Kecipir (*Psophocarpus tetragonolobus L.*). *Pro Food (Jurnal Ilmu Dan Teknologi Pangan*, *5*(2), 486–495.

Barus, T., Salim, D. P., & Hartanti, A. T. (2019). Kualitas Tempe menggunakan *Rhizopus delemar TB 26* dan *R. delemar TB 37* yang Diisolasi dari Inokulum Tradisional Tempe "daun waru." *Jurnal Aplikasi Teknologi Pangan*, *8*(4), 143–148.

Budiono, R. A. (2016). *Pengaruh Jenis Kapang terhadap Aktivitas Fermentasi Tempe Saga Pohon (Adenanthera pavonina L.)* [Skripsi]. Jakarta: Fakultas Sains dan Teknologi, Universitas Islam Negeri Syarif Hidayatullah.

Kemit, N., Widarta, I. W. R., & Nocianitri, K. A. (2016). Pengaruh Jenis Pelarut dan Waktu Maserasi terhadap Kandungan Senyawa Flavonoid dan Aktivitas Antioksidan Ekstrak Daun Alpukat (*Persea Americana Mill*). *Jurnal Ilmu Dan Teknologi Pangan*, *5*(2), 130–141.

Laksono, A. S., Marniza, & Rosalina, Y. (2019). Karakteristik Mutu Tempe Kedelai Lokal Varietas Anjasmoro dengan Variasi Lama Perebusan dan Penggunaan Jenis Pengemas. *Jurnal Agroindustri*, *9*(1), 8–18.

Lestari, S. R., Witjoro, A., & Poejiani, S. (2018). The Potential of Single Garlic Oil in Inhibiting The Growth and Damaging The Membrane of *Pseudomonas aeruginosa* Bacteria. *Journal of Tropical Biodiversity and Biotechnology*, *3*(3), 67–72. https://doi.org/10.22146/jtbb.33498

Lisiswanti, R., & Haryanto, F. P. (2017). Allicin pada Bawang Putih (*Allium sativum*) sebagai Terapi Alternatif Diabetes Melitus Tipe 2. *Majority*, *6*(2), 31–36.

Mawaddah, N., Fakhrurrazi, & Rosmaidar. (2018). Aktivitas Antibakteri Ekstrak Tempe Terhadap Bakteri *Staphylococcus aureus*. *Jurnal Ilmiah Mahasiswa Veteran*, *2*(3), 230–241.

Moulia, M. N., Syarief, R., Iriani, E. S., & Kusumaningrum, H. D. (2018). Antimikroba Ekstrak Bawang Putih. *Jurnal Pangan*, *27*(1), 55–66.

Noviana, A., Dieny, F. F., Rustanti, N., Anjani, G., & Afifah, D. N. (2018). Antimicrobial Activity of Tempeh Gembus Hydrolyzate. *IOP Conference Series Earth and Environmental Science*, *116*(1), 1–7.

Pratama, M. (2017). Identifikasi Atribut dan Rasa Rempah dengan Profiled Test. *Jurnal Agroindustri Halal*, *3*(2), 126–132.

Pratiwi, R. H. (2017). Mekanisme Pertahanan Bakteri Patogen terhadap Antibiotik. *Jurnal Pro-Life*, *4*(3), 418–429.

Purwantiningsih, T. I., Rusae, A., & Freitas, Z. (2019). Uji In Vitro Antibakteri Ekstrak Bawang Putih sebagai Bahan Alami untuk Menghambat Bakteri *Staphylococcus aureus* dan *Escherichia coli*. *Sains Peternakan*, *17*(1), 1–4.

Purwanto, Y. A., & Weliana. (2018). Kualitas Tempe Kedelai pada Berbagai Suhu Penyimpanan. *Journal of Agro-Based Industry*, *35*(2), 106–112.

Rahman, M. S. (2014). Allicin and Other Functional Active Components in Garlic : Health Benefits and Bioavailibility. *International Journal of Food Properties*, *10*(2), 245–268.

Rahmi, S. L., Mursyid, & Wulansari, D. (2018). Formulasi Tempe Berbumbu serta Pengujian Kandungan Gizi. *Industria : Jurnal Teknologi Dan Manajemen Agroindustri*, *7*(1), 57–65.

Salima, J. (2015). Antibacterial Activity of Garlic (*Allium sativum l.*). *MAJORITY*, *4*(2), 30–39.

Soraya, C., Chismirina, S., & Novita, R. (2018). Pengaruh Perasan Bawang Putih (*Allium sativum L.*) sebagai Bahan Irigasi Saluran Akar Dalam Menghambat Pertumbuhan *Enterococcus faecalis* Secara In Vitro. *Cakradonya Dental Journal*, *10*(1), 1–9.

Sudjatini. (2020). Pengaruh Cara Pengolahan terhadap Aktivitas Antioksidan Ekstrak Bawang Putih (*Allium sativum L.*) Varietas Kating dan Sinco. *Agrotech Jurnal Ilmiah Teknologi Pertanian*, *3*(1), 1–7.

Wiryadana, K. A., Pinatih, K. J. P., & Hendrayana, M. A. (2019). Uji Daya Hambat Kombinasi Siproflaksasin dengan Obat Non - Antibiotik Artesunat, Diklofenak dan Loperamid terhadap Pertumbuhan Isolat Klinis *Escherichia coli*. *E-Jurnal Medika*, *8*(3), 1–8.

Zhafira, R. (2018). Pengaruh Lama Aging terhadap Sifat Fisik, Kimia, dan Aktivitas Antioksidan Produk Bawang Hitam Lanang. *Jurnal Pangan Dan Agroindustri*, *6*(1), 34–42.