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Antioxidant and Antibacterial Activities Enhancement of Solid-state Fermented Candlenut Kernels by *Aspergillus oryzae*

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According to several studies, solid state fermentation (SSF) can enhance antioxidant and antibacterial activity of natural sources, and microorganism that is widely used in this kind of research is *Aspergillus oryzae*. Therefore, this study employed SSF by *A. oryzae* to enhance antioxidant and antibacterial activity of candlenut kernel. Candlenut kernel powder, that has been moistened with 60% water, was inoculated with 10% (w/w) of 5-day-culture of *A. oryzae,* and was fermented for 9 days (until exponential phase; sample-1) and 12 days (until stationary phase; sample-2). The fermented candlenut kernels was extracted by ethanol and concentrated using rotary evaporator. Total phenolic content of control (unfermented extract), sample-1, and sample-2 are 0.183 mg GAE g^{-1}, 2.761 mg GAE g^{-1}, and 4.194 mg GAE g^{-1}, respectively. This results supported the IC_{50} value determined by DPPH (2,2-diphenyl-1-picrylhydrazyl) method, those are 617.11 μg mL^{-1}, 260.23 μg mL^{-1}, and 45.29 μg mL^{-1}. These results revealed a very strong antioxidant activity (< 50 μg mL^{-1}) in the sample fermented until stationary phase. Antibacterial assay against *Staphylococcus aureus* resulted diameter of inhibition zone 7.17 mm, 13.51 mm, and 18.51 mm, respectively; whereas against *Pseudomonas aeruginosa* resulted diameter of inhibition zone 6.52 mm, 11.786 mm, and 15.269 mm, respectively. From this result, SSF until stationary phase enhanced higher antioxidant and antibacterial activity compared the other treatments.

Key words: antibacterial, antioxidant, *Aspergillus oryzae*, solid state fermentation, stationary phase

Berdasarkan beberapa penelitian, *solid state fermentation* (SSF) dapat meningkatkan aktivitas antioksidan dan antibakteri sumber hayati, dan mikroorganisme yang sering digunakan dalam penelitian seperti ini adalah *Aspergillus oryzae*. Oleh karena itu, dalam penelitian ini dilakukan SSF oleh *A. oryzae* untuk meningkatkan aktivitas antioksidan dan antibakteri biji kemiri. Serbuk biji kemiri, yang dilembabkan dengan 60% air, diinokulasikan dengan 10% kultur *A. oryzae* berumur 5 hari, dan difermentasi selama 9 hari (hingga fase eksponensial; sampel-1) dan selama 12 hari (hingga fase stasioner; sampel-2). Biji kemiri yang telah difermentasi, diekstraksi menggunakan etanol dan dikonsentrasikan menggunakan *rotary evaporator*. Kandungan fenolik total sampel kontrol (tanpa fermentasi), sampel-1, dan sampel-2 berturut-turut adalah 0,183 mg GAE g^{-1}, 2,761 mg GAE g^{-1}, dan 4,194 mg GAE g^{-1}. Hasil ini mendukung nilai IC_{50} yang ditentukan menggunakan metode DPPH (2,2-diphenyl-1-picrylhydrazyl), yaitu secara berturut-turut 617,11 μg mL^{-1}, 260,23 μg mL^{-1}, and 45,29 μg mL^{-1}. Hasil ini menunjukkan aktivitas antioksidan yang sangat kuat (< 50 μg mL^{-1}) pada sampel yang difermentasi hingga fase stasioner. Uji antibakteri terhadap *Staphylococcus aureus*, secara berturut-turut, menghasilkan diameter zona inhibisi sebesar 7,17 mm, 13,51 mm, dan 18,51 mm, sedangkan terhadap *Pseudomonas aeruginosa* menghasilkan diameter zona inhibisi sebesar 6,52 mm, 11,786 mm, dan 15,269 mm. Berdasarkan hasil penelitian ini, dapat disimpulkan bahwa SSF hingga fase stasioner dapat meningkatkan aktivitas antioksidan dan antibakteri lebih tinggi dibandingkan perlakuan yang lain.

Kata kunci: antibakteri, antioksidan, *Aspergillus oryzae*, pertumbuhan fase stasioner, *solid state fermentation*

Candlenut is plant that distributed widely in the world. Parts of candlenut tree, such as leaf, seed, pericarp, sap, and flower can be utilized as natural herbal medicine. Its periderm is used for curing diarrhaea and tumour. Candlenut leaves for curing headache, fever, boils, arthritis, and gonorrhea. Flower and fresh sap for curing sprue in children. Candlenut seed contents sterol, flavonoids, and triterpene that act as anti-inflammation, anti-pyretic, and is traditionally used for wound. Therefore, candlenut plant can be utilized as medicine, especially due to its extract that has antioxidant and antibacterial activities (Ako *et al*. 2005; Krisnawati *et al*. 2011).

Based on the research that has already been conducted (Siddique *et al*. 2011), IC_{50} value of candlenut seed antioxidant activity using DPPH method is 30.37 mg mL^{-1} compared to IC_{50} value of BHT standard 15.3 μg mL^{-1}. Both DPPH (2,2-diphenyl-1-picrylhydrazyl) and BHT (butylated hydroxytoluene)

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are stable radical compounds that can reduce chemical reaction of other radical compounds, therefore both compounds are usually used in antioxidant activity assay. Based on research which was conducted by Pakpahan & Pangaribuan (2018), antibacterial activity of ethanol candlenut kernel extract to *Staphylococcus aureus* resulted 19.17 mm of inhibition zone (strong inhibition response) and to *Pseudomonas aeruginosa* resulted 6-8 mm of inhibition zone (moderate inhibition response). Based on those researches, antioxidant and antibacterial activity of candlenut kernel are possible to be increased. Several researches proved that antioxidant and antibacterial activity of natural sources can be enhanced after fermented using solid state fermentation method (Ako *et al*. 2005, Juan and Chou 2010). Solid state fermentation can also increase production rate, decrease production cost, and using more simple technique compared to submerged fermentation (Juan and Chou 2010). Microorganisms that generally used in solid state fermentation are *Aspergillus oryzae*, *Aspergillus niger*, *Monascus purpureus*, and *Bacillus subtilis* (Das and Mukherjee 2007; Juan and Chou 2010; Wen *et al*. 2013; Jamaluddin *et al*. 2016). The objective of this research is to increase the activity of antioxidant and antibacterial in solid state fermented candlenut kernels by *Aspergillus oryzae*.

MATERIALS AND METHODS

Substrate Preparation. Candlenut was peeled, washed with water, sun-dried, and the kernels were pounded into powder.

Microorganisms Preparation. *Aspergillus oryzae* was purchased from Laboratory of Microbiology, Sekolah Ilmu dan Teknologi Hayati (SITH), Institut Teknologi Bandung. *A. oryzae* was rejuvenated on potato dextrose agar (PDA). Inoculum was cultured on growth media yeast peptone glycerol (YPG) and was incubated in 37°C for 72 h. Culture on YPG media will be used to make standard curve, growth curve, and to ferment candlenut powder (Lee *et al*. 2008). *Pseudomonas aeruginosa* and *Staphylococcus aureus* were rejuvenated in *nutrient broth* (NB) and incubated in 37°C for 24 h (Pakpahan and Pangaribuan 2018).

Solid State Fermentation. As much as 50 g candlenut powder was moistened with 30 mL distilled water until approximately 60% of humidity in fermentation flask. The fermentation flask was autoclaved for 15 min and cooled until 25°C before inoculated with *A. oryzae*. As much as 10% (v/v) of 5 days *Aspergillus oryzae* was inoculated into fermentation media, whereas for control is fermentation media without *A. oryzae*. Fermentation flask was incubated in 37°C. Fermentation was ended when reached exponential phase time (day-9) or stationary phase time (day-12). Fermentation was repeated three times to achieve accurate data.

Extraction. As much as 100 mL of 96% ethanol was added into each fermentation flasks, stirred using magnetic stirrer for 24 h in 25°C. Extracts were filtered using gauze and concentrated in vacuum evaporator. The concentrated extracts were determined for its total phenolic content, antioxidant, and antibacterial activity.

Total Phenolic Determination. Total phenolic determination was conducted based on modification method in Juan and Chou (2010). As much as 0.1 mL of extract aliquot was mixed with 1 mL Folin-Ciocalteau reagent and left for 3 min reaction. Three hundred µL of 1N Na_2CO_3 was added and left for 90 min reaction in 25°C. Absorbance was measured on 725 nm. Gallic acid standard curve was determined using 8 concentrations of Gallic acid ranged from 0 until 100 ppm. As much as 0.01 g Gallic acid in 100 mL volumetric flask was added with 1 mL ethanol, and then ethanol was added again until the indicator line. From 100 ppm stock solution was taken 1 mL and added with 1 mL reagen Folin-Ciocalteu in 10 mL volumetric flask. The solution was homogenized and left for several minutes. Then 4 mL of 1N Na_2CO_3 was added into volumetric flask and left for 15 min in 25°C. Solution absorbance was measured on 725 nm. Absorbance values of Gallic acid were plotted to Gallic acid concentrations, and count for Gallic acid standard curve equation. Total phenolic content will be in µg Gallic acid/mg extract.

Antioxidant Activity Assay. Antioxidant activity was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method (Tristantini *et al*. 2016). Every 10 mg sample was dissolved in 100 mL ethanol. Dissolved sample was diluted into 5 concentration, those are 50 ppm, 100 ppm, 150 ppm, 200 ppm, and 250 ppm. Two mL of every diluted sample was mixed with 2 ml of 50 ppm DPPH. Two mL of ethanol was mixed with 2 mL of 50 ppm DPPH as blank. Both solution were incubated in 25°C for 30 min. Absorbance was measured on 517 nm. Percent inhibition of DPPH will be counted using the following equation:

$$\%inhibition = \frac{\text{Blank absorbance} - \text{sample absorbance}}{\text{Blank absorbance}} x\ 100$$

***In vitro* Antibacterial Activity Assay.** Antibacterial activity assay was conducted using agar disk diffusion method (Pakpahan and Pangaribuan 2018). As much as 14.0449 g nutrient agar (NA) media was dissolved into 500 mL distilled water, poured into 1000 mL Erlenmeyer flask. NA media was autoclaved for 15 min. Sterilized media was poured into 6 Petri dishes and cooled at 25°C. Antibacterial activity was assayed to *Staphylococcus aureus* and *Pseudomonas aeruginosa*. One hundred l bacterial suspension of *Staphylococcus aureus* and *Pseudomonas aeruginosa* from exponential phase were inoculated to sterilized NA media and spread using glass rod spreader. Sterilized disk papers were placed on NA agar and each disk paper was dripped with 10 µl sample, ampicillin (positive control), or ethanol (solvent). NA media were incubated at 37°C for 24 h. Antibacterial activity was observed by measuring the diameter of inhibition zone around disk paper using calipers. Ethanol showed no antibacterial activity, whereas ampicillin showed a very strong antibacterial activity with inhibition zone diameter of 36 mm to *S. aureus* and 32.78 mm to *P. aeruginosa*.

RESULTS

Total phenolic content was increase after candlenut kernels were solid state fermented by *Aspergillus oryzae* until exponential phase (2.76 mg GAE g^{-1}) and the highest total phenolic content was reached when fermentation was stopped at stationary phase (4.19 mg GAE g^{-1}), as showed in Table 1.

Antioxidant activity was also increase with the increasing of total phenolic content, as showed in Table 2. IC_{50} of stationary-phase fermented extract was 45.29 ppm, it was 13.6 times higher than the unfermented extract (617.11 ppm), and 5.7 times higher than the exponential-phase fermented extract (260.23 ppm). Stationary-phase fermented extract had a very strong activity (IC_{50} < 50 ppm), whereas exponential-phase fermented extract and unfermented extract had a weak activity (IC_{50} > 200 ppm).

The fermented candlenut kernel extracts showed a strong inhibition response compared to unfermented extract (Table 3), both to *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The stationary-phase fermented extracts showed wider inhibition zone (18.51 mm and 15.26 mm) compared to exponential-phase fermented extract (13.51 mm and 11.79 mm) respectively to *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

DISCUSSION

Solid state fermentation (SSF) made phenolic compound extraction from natural sources more easier (Dey and Kuhad 2014). The phenolic compounds will be converted into a more soluble form by the enzyme of microorganism. *Aspergillus oryzae* was the microorganism which was used in this research, and was predicted can convert phenolic compound in candlenut kernels into soluble form during the fermentation process. The prediction was proved in this research, with the increasing content of total phenol in the fermented extract. Based on research conducted by Rashid *et al*. (2019), it was stated that after solid state fermentation by *A. oryzae*, the total phenolic content of rice bran was increase 3.8-fold higher than the unfermented. And three types of phenolic acid detected by HPLC in unfermented rice bran (coumaric, ferulic acid, and protocatechuic) was also increase in the fermented rice bran by 3.2-fold, 52-fold, and 3.2-fold respectively. The increasing content of ferulic acid in solid-state fermented rice bran by *Rhizopus oryzae* was only 23.2-fold compared to the unfermented rice bran (Schmidt *et al*. 2014). And the total phenolic content of solid-state fermented rice bran by *Monascus purpureus* was only increase 1.04-fold higher than the unfermented (Jamaluddin *et al*. 2016). SSF method has an ability to produce secondary metabolites more efficient than SmF (submerged fermentation) method and ideal for fungal cultivation which can grow at limited water. The limited water need gives SSF some advantages, such as reduced downstream processing and reduced stirring, therefore SSF can produce high concentration product in a lower requirement of energy (Nigam 2009). Martins *et al*. (2011) stated that phenolic compounds, that often be extracted from solid-state fermented substrate, were flavonoid, flavonol, flavone, flavanol, isoflavon, and anthocyanidin. Phenolic compounds were considered as natural antioxidant and represent several important bioactive groups in foodstuff. These compounds consist in all plants which were used as foodstuff, but the types and concentration were vary depend on the plant species, genetic factors, and environmental condition. Solid state fermentation process is often used to increase the number of phenolic compound in several foodstuff and to increase antioxidant activity which related to the increasing of total phenolic compound.

Several methods have already been conducted to increase the synthesis of bioactive metabolite from

Table 1. Total phenolic content

Sample	Total phenolic content (mg GAE g^{-1})
Unfermented	0.18
Fermented until exponential phase	2.76
Fermented until stationary phase	4.19

Table 2. IC_{50} value and antioxidant activity level

Sample	IC_{50} (ppm)	Antioxidant activity
Unfermented	617.11	Very weak
Fermented until exponential phase	260.23	Very weak
Fermented until stationary phase	45.29	Very strong

Table 3. Antibacterial activity of candlenut kernel extracts to *Staphylococcus aureus* and *Pseudomonas aeruginosa*

Sample	*Staphylococcus aureus*		*Pseudomonas aeruginosa*	
	Inhibition zone	Inhibition Response	Inhibition zone	Inhibition Response
Unfermented	7.17 mm	Moderate	6.52 mm	Moderate
Fermented until exponential phase	13.51 mm	Strong	11.79 mm	Strong
Fermented until stationary phase	18.51 mm	Strong	15.26 mm	Strong

microbes (Schmidt *et al*. 2014), one of those is solid state fermentation. Phenolic compound in plants functioned as protecting mechanism and other biological function such as antioxidant activity. Plants produce several bioactive compounds which have strong correlation between its antioxidant activity and its total phenolic content, and phenolic compounds have the biggest contribution to antioxidant activity (Li *et al*. 2008). The very strong antioxidant activity of stationary-phase fermented extract was supported by its highest content of total phenolic compounds. Phenolic compounds are secondary metabolites which were produced in the end of exponential phase and during the stationary phase (Madigan *et al*. 2012). During the fermentation process, extracellular enzyme of fungi was produced to release the phenolic group from substrate matrix together with the production of phenolic compound during secondary metabolism (Dey *et al*. 2016), and also converted phenolic compound into a more soluble form in ethanol (Dey and Kuhad 2014). Siahaan *et al*. (2015) stated that the inhibition zone diameter of fermented extract in ethanol related with the components which can be extracted by ethanol. Ethanol is a polar compound that is able to extract other polar compounds such as phenol, saponin, alkaloid, and terpenoid. Half of those compounds have antioxidant and antibacterial activities.

The wider inhibition zone diameter in antibacterial assay to *Staphylococcus aureus* compared to *Pseudomonas aeruginosa,* was caused by the different Gram characteristic between both bacteria. *Staphylococcus aureus* is a gram-positive bacteria which its cell wall has 80 nm of peptidoglycan thickness, but has no outer membrane (Al Hanif 2009). Madigan *et al*. (2012) stated that the number of peptidoglycan layers in Gram-positive bacteria is 40 layers, which composed 90% of all cell wall components. *Pseudomonas aeruginosa* is a Gram-negative bacteria which its cell wall has 10% peptidoglycan layers of all cell wall components. Cell wall of Gram-negative bacteria is composed mostly by outer membrane which made its cell wall more complex. Action mechanism of most antibacterial compound is by inhibit the biosynthesis of peptidoglycan in the transpeptidase reaction which will cause lysis of cells. In general, antibacterial compound is difficult to pass the outer membrane and attack peptidoglycan in the cell wall of Gram-negative bacteria because of the different polar characteristic.

The similar profile of the increasing antioxidant and antibacterial activity with the increasing of total phenolic content in the stationary-phase and exponential-phase fermented extracts compared to

unfermented extract, is most likely affected by the activity of β-glucosidase enzyme which convert phenolic isoflavon compound in candlenut kernels into free isoflavon called aglycones. Aglycones have an effective biological activity to inhibit chronic disease such as cardiovascular and cancer. Beta-glucosidase enzymes able to hydrolyze β-glucoside bonds of carbohydrate conjugates in phenolic compounds. The enzymatic hydrolysis on β-glucoside bonds of phenolic compounds seemed a promising method to increase the concentration of free polyphenol and increase the nutraceutical activity in several natural sources (Georgetti *et al*. 2009). Besides that, as stated by Widowati *et al*. (2011), the activity of β-glucosidase to hydrolyze β-glucoside of carbohydrate conjugates will produce carbohydrates in the form of monosaccharides or disaccharides as nutrition to support *A. oryzae* growth.

In conclusion, the present study has shown that solid state fermentation of candlenut kernels until stationary phase time by *Aspergillus oryzae* can enhance the production of phenolic compounds and therefore increase the antioxidant and antibacterial activities.

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REFERENCES

Ako H, Kong N, Brown A. 2005. Fatty acid profiles of kukui nut oils over time and from different sources. Ind Crop Prod. 22(2): 169-174. doi: 10.1016/j.indcrop.2004.07.003.

Al Hanif MS. 2009. Pola Resistensi Bakteri dari Kultur Darah terhadap Golongan Penisilin di Laboratorium Mikrobiologi Klinik Fakultas Kedokteran Universitas Indonesia (LMK-FKUI) Tahun 2001 – 2006. Bacterial resistance pattern from blood isolates against penicillins in Clinical Microbiology Laboratory FMUI Year 2001-2006. [minithesis]. Jakarta: Universitas Indonesia.

Das K, Mukherjee AK. 2007. Comparison of lipopeptide biosurfactants production by *Bacillus subtilis* strains in submerged and solid state fermentation systems using a cheap carbon source: some industrial applications of biosurfactants. Process Biochemistry 42(8):1191-1199. doi: 10.1016/ j.procbio.2007.05.011.

Dey TB, Chakraborty S, Jain KK, Sharma A, Kuhad RC. 2016. Antioxidant phenolics and their microbial production by submerged and solid state fermentation process: A review. Trends in Food Science & Technology 53:60-74. doi: 10.1016/j.tifs.2016.04.007.

Dey TB, Kuhad RC. 2014. Enhanced production and extraction of phenolic compounds from wheat by solid-state fermentation with *Rhizopus oryzae* RCK2012. Biotechnology Reports 4:120-127. doi: 10.1016/j.btre.2014.09.006.

Georgetti SR, Vicentini FTMC, Yokoyama CY, Borin MF, Spadaro ACC, Fonseca MJV. 2009. Enhanced *in vitro* and *in vivo* antioxidant activity and mobilization of free phenolic compunds of soybean flour fermented with different beta-glucosidase-producing fungi. Journal of Applied Microbiology 106:459-466. doi: 10.1111/j.1365-2672.2008.03978.x.

Jamaluddin A, Razak DLA, Rashid NYA, Sharifudin SA, Kahar AA, Saad AZM, Long K. 2016. Effects of solid state fermentation by *Monascus purpureus* on phenolic content and biological activities of coconut testa and rice bran. Jurnal Teknologi (Sciences & Engineering) 78(11-2):23-28. doi: 10.11113/jt.v78.9939.

Juan MY, Chou CC. 2010. Enhancement of Antioxidant Activity, Total Phenolic and Flavonoid Content of Black Soybeans by Solid State Fermentation with *Bacillus subtilis* BCRC 14715. Food Microbiol. 27(5):586-591. doi: 10.1016/j.fm.2009.11.002.

Krisnawati H, Kallio M, Kanninen M. 2011. *Aleurites moluccana* (L.) Willd: Ecology, silviculture and productivity. CIFOR, Bogor, Indonesia.

Lee IH, Hung YH, Chou CC. 2008. Solid state fermentation with fungi to enhance the antioxidative activity, phenolic and anthocyanin contents of black bean. Int J Food Microbiol. 121(2):150-156. doi: 10.1016/j.ijfoodmicro.2007.09.008.

Li HB, Wong CC, Cheng KW, Chen F. 2008. Antioxidant properties in vitro and total phenolic contents in methanol extracts from medicinal plants. LWT-Food Science and Technology 41(3):385-390. doi: 10.1016/j.lwt.2007.03.011.

Madigan M, Martinko JM, Stahl DA, Clark DP. 2012. Brock Biology of Microorganisms. 13th ed. Netherlands: Pearson.

Martins S, Mussatto SI, Avila GM, Saenz JM, Aguilar CN, Teixeira JA. 2011. Bioactive phenolic compounds: Production and extraction by solid-state fermentation. A review. Biotechnol Adv. 29(3):365-373. doi: 10.1016/j.biotechadv.2011.01.008.

Nigam PS. 2009. Production of bioactive secondary metabolites. In: Biotechnology for agro-industrial residues utilisation. Nigam PS, Pandey A. Eds. Springer. doi: 10.1007/978-1-4020-9942-7.

Pakpahan NL, Pangaribuan N. 2018. Evaluasi Potensi Ekstrak Minyak Kemiri (*Aleurites molucana*) yang diekstrak menggunakan metode gabungan Maserasi dan Soxhletasi, sebagai Antibakteri terhadap *Staphylococcus aureus* dan *Pseudomonas aeruginosa*. Potential

evaluation of candlenut oil *(Aleurites moluccana*) extracted by maceration and soxhletation combination methods as antibacterial against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. [minithesis]. Laguboti: Institut Teknologi Del.

Rashid NYA, Ghani AA, Jamaluddin A, Razak DLA. 2019. Quantification of phenolic compounds changes by *Aspergillus oryzae* on rice bran fermentation. Food Research 3(2):133-137. doi: 10.26656/fr.2017.3(2).122.

Schmidt CG, Goncalves LM, Prietto L, Hackbart HS, Furlong EB. 2014. Antioxidant activity and enzyme inhibition of phenolic acids from fermented rice bran with fungus *Rhizopus oryzae*. Food Chem. 146:371-377. doi: 10.1016/j.foodchem.2013.09.101.

Siahaan MR, Alimuddin AH, Harlia. 2015. Identifikasi Metabolit Sekunder Ekstrak Landak Laut (*Diadema setosum*) Dan Uji Aktivitas Antibakteri *Escherichia coli* dan *Staphylococcus aureus*. Jurnal Kimia Katulistiwa 4(4):53-60.

Siddique BM, Ahmad A, Alkarkhi A, Ibrahim MH, Omar M. 2011. Chemical Composition and Antioxidant Properties of Candlenut Oil Extracted by Supercritical CO_2. Journal of Food Science 76(4):C535-C542. doi: 10.1111/j.1750-3841.2011.02146.x.

Tristantini D, Ismawati A, Pradana BT, Jonathan JG. 2016. Pengujian Aktivitas Antioksidan Menggunakan Metode DPPH pada Daun Tanjung (*Mimusops elengi* L). Pengembangan Teknologi Kimia untuk Pengolahan Sumber Daya Alam Indonesia. Prosiding Seminar nasional Teknik Kimia Kejuangan; 2016 Mar 17. Yogyakarta. p G1-1-G1-7.

Wen YL, Yan LP, Chen CS. 2013. Effects of fermentation treatment on antioxidant and antimcrobial activities of four common Chinese herbal medicinal residues by *Aspergillus oryzae*. Journal of Food and Drug Analysis 21:219-226. doi: 10.1016/j.jfda.2013.05.013.

Widowati E, Andriani MAM, Kusumaningrum AP. 2011. Study of total probiotics bacteria and antioxidant activity in yoghurt tempeh using substrate variation. Jurnal Teknologi Hasil Pertanian IV(1): 18-31.