Antioxidant activity of soybean and *gembus* tempeh

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ABSTRACT

Soybean tempeh and *gembus* tempeh are traditional foods that have long been known in Indonesia, which in a modern way are classified as functional food. Various studies related to the antioxidant activity of soybean and *gembus* tempeh has been reported. This study aimed to compare the antioxidant capacity of soybean tempeh and *gembus* tempeh that are circulating in the community. The results of the research are expected to be an evaluation of soybean tempeh and *gembus* tempeh quality available in the market. The results of this research are expected to be an evaluation of soybean tempeh and *gembus* tempeh quality available in the market. The research design was a cross-sectional experimental study to measure antioxidants activity of 31 soybeans tempeh and 29 *gembus* tempeh. Sample of this study was selected through simple random sampling technique. The measurement of antioxidant activity carried out was the 2,2-diphenyl 1-picrylhydrazyl (DPPH) method; ethanol extraction of 95%. Results revealed that the antioxidant activity of *gembus* tempeh was significantly higher than soybean tempeh; (32.521; 19.831) vs., (17.016; 13.195), respectively.

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1. INTRODUCTION

Tempeh is a traditional food has been known in Indonesia. The first reference Tempeh was found in 1875. Serat Centini (the manuscript from Java in the early 19th century) has found the word tempeh, e.g. Jaesantenempeh (a dish of tempeh with coconut milk) and Kadheletempehsrudeng [1, 2]. The words showed that tempeh has been known as an eating habit of Javanese for centuries, especially at Yogyakarta and Surakarta [3-5]. This is evidence that tempeh is a kind of food which have been often consumed by the people of Indonesia, especially the Javanese.

Indonesia is the biggest producer of tempeh in the world and it can be the biggest market of soybean in Asia. As much as 50% of Indonesian soybean consumption is used to produce tempeh, 40% tofu and 10% in other (such as tauco, soy sauce). The average tempeh consumption in Indonesia is currently estimated at around 6.45 kg per person [3].

Indonesia has various types of tempeh; each region has a different type of tempeh based on the raw material used. For example, menjes tempeh from East Java is made from tofu dregs and peanuts; benguk
tempeh from Yogyakarta is made from Koro Benguk (Mucunapruriens) and lamtoro tempeh (Laucaenaglau). The common varieties of tempeh are soybean tempeh and *gembus* tempeh [6].

In contrast to soybean tempeh, the historical record of *gembus* tempeh was obtained from Gandjar [7]. It is said that the history of *gembus* tempeh begins in 1943. At that time, there was a food crisis in Java. The community utilizes tofu dregs from the factory, which are commonly used for animal feed to be processed into food such as tempeh [7]. Nowadays, *gembus* tempeh is still considered as lower caste food because of its ingredients. Even in Solo, there is a food made from *gembus* tempeh, which is called “kere satay.” The meaning is satay for poor people.

Based on the product characteristics, soybean tempeh is higher in protein content than that of *gembus* tempeh because it directly fermented from the soybean seeds. The protein content of soybean tempeh is 37.10-41.79 per 100 grams [8]; whereas that of *gembus* tempeh is only 6.7 grams per 100 grams [9]. The low-level protein of *gembus* tempeh made people underestimate it because it was considered as an innutritious food, although *gembus* tempeh is usually eaten as a side dish or as a snack [10]. Underestimated people’s perception of *gembus* tempeh was not entirely correct.

Study on *gembus* tempeh in Indonesia conducted by Agustina *et al.*, showed that antioxidants level with DPPH was 52.2±6.7% [11]. The other study by Diana reported that *gembus* were shown having proteolytic, fibrinolytic; antioxidant and antimicrobial activities may provide a significant opportunity for health [12, 13].

Study about soybean tempeh conducted by Purwoko, reported that the is flavone rate in soybean tempeh was 864.38 µg/g [14]. The study of Chang *et al* about DPPH radical scavenging effect in soybean tempeh showed that level IC50 with water extract and 10 days of incubation time in 9.7 mg/ml, it was a moderate level [15]. The is flavone rate in soybean tempeh and *gembus* tempeh were influenced by multiple factors, such as the variety of microorganism, soybean quality, incubation time and so on [16]. Nonetheless, the scientific evidence still appears to be limited as most of them are derived from laboratory research, tempeh was made in laboratory [11, 14, 15] while the research of tempeh sold in traditional market was limited.

This present study intends to determine the antioxidant activity of the soybean tempeh and *gembus* tempeh which available at the traditional market. Soybean tempeh and *gembus* tempeh that are sold in traditional market are made with different ingredients and manufacturing processes, so that it can produce products with different qualities. This study will to know about quality soybean tempeh and *gembus* tempeh from antioxidant activity. The result of this study will be used as information to the producers of tempeh in traditional market to develop the quality of their products.

2. RESEARCH METHOD

The design study is a cross-sectional study [17, 18]. In this study, the researchers intend to determine antioxidant activity of the soybean tempeh and *gembus* tempeh in traditional market of Sukoharjo and Karanganyar. The total samples are 31 soybean tempeh and 29 *gembus* tempeh was purchased randomly [19] from soybean tempeh merchant and *gembus* tempeh merchant of Bengkonang, Glondongan, Sukoharjo, Bejen and Palur markets. The procurement of tempeh in the fresh condition with incubation time 42 hours.

2.1. Preparation of tempeh extract

Tempeh was dried in an oven at temperatures of 55-66°C [20]. Drying time of soybean tempeh was 8-9 hours, while *gembus* tempeh 7-8 hours. Tempeh powder (100 gram) was mixed with 100 ml of ethanol 95%. Macerated for three days, filtered with filter paper [21, 22], modified. The extract was dried in a water bath until it changes into a paste.

2.2. Antioxidant testing procedure

The antioxidant analysis in this research is the DPPH method ((2,2-diphenyl 1-picrylhydrazyl). Determinations relying on photometric measurements are simple, rapid and inexpensive [23, 24]. The test procedure was; make 0.15mM by dissolving 20 mg DPPH in 10 ml ethanol 95%, put in a 50 ml volumetric flask, shaken until homogeneous. The next step is determining the maximum wavelength with an absorbance of 450-600 nm, determine the operating time. Sample solutions is created by dissolving 50 mg of the sample extract in 10 ml ethanol for 15 minutes, then put in a 10 ml volumetric flask, then add ethanol to the limit, then homogenized. The homogeneous sample solution is inserted in a 5 ml flask, 1 ml of DPPH solution is added, and then homogenized, and then incubates in the dark room for 20 minutes, and measure the absorbance at a wavelength of 516.5 nm.
3. RESULTS AND DISCUSSION

There were three types of tempeh packaging, polyethylene bag (PB), banana leaf (BL), and the combination of both (PBBL). The most of gembus tempeh were wrapped by polyethylene bag. As shown in Table 1 the mean of absorbance of soybean tempeh based on the packaging, combination of polyethylene bag and banana leaf (PBBL) is the highest score followed by PB and BL. Soybean tempeh PB is the most common in the traditional market, has an antioxidant activity level 18.65%, two times greater than tempeh BL as shown in Table 1. This result similar to research conducted by Hashim that compared two materials packaging, polyethylene bag and banana leaf. The result showed that tempeh which wrapped by polyethylene bag enhanced the polyphenolic components released through the fermentation process [25]. In current study, tempeh which wrapped by combination of banana leaves and polyethylene bag (PBBL) has a higher antioxidant activity value than PB and BL. According to Santhirasegaram’s research, the materials used for packaging did not induce any significant alteration in antioxidant properties of tempeh, but it was significant to the shelf life of tempeh [26].

| Packaging | n  | The mean of absorbance (%) | SD  | Minimum score | Maximum score |
|-----------|----|----------------------------|-----|---------------|---------------|
| PB        | 22 | 18.65                      | 13.412 | 2.9           | 54.32         |
| BL        | 6  | 8.67                       | 8.001  | 2.05          | 24.07         |
| PBBL      | 3  | 21.69                      | 15.581 | 4.65          | 35.21         |

Gembus tempeh available in the traditional market mostly wrapped by polyethylene bag (PB). There were only three samples of PBBL packaging in the market. Based on the Table 2, the highest of antioxidant activity when the gembus tempeh wrapped by polyethylene bag (PB) (32.84%) than combination of polyethylene bag and banana leaf (PBBL) (29.778%).

| Packaging | n  | The mean of absorbance (%) | SD  | Minimum score | Maximum score |
|-----------|----|----------------------------|-----|---------------|---------------|
| PB        | 26 | 32.84                      | 20.852 | 5.28          | 80.05         |
| PPBL      | 3  | 29.78                      | 7.670  | 23.17         | 38.19         |

There are many factors that influence antioxidant activity level, including the differences of processing tempeh, the type of microorganism, incubation time, and the differences of density gembus tempeh and soybean tempeh [2]. Referred to Widoyo’s study, 2010 incubation time affect the antioxidant tempeh. In 42 hours, fermented soybean tempeh showed 67.5% antioxidant activity [27]. Similar to Ariani’s study that 72 hours fermentation gave a higher absorbance percentage compared to 48 hours fermentation in soybean tempeh [28]. Nurrahman’s research which assessed antioxidant activity in various inoculums showed tempeh fermented with R. Stolonifer showed the highest antioxidant activity [16]. Further research needs to be done to be able to assess the differences in the tempeh production, microorganism type, incubation time and the packaging of tempeh to the antioxidant activity.

Figure 1 shows the value of absorbance (%) on soybean tempeh and gembus tempeh, there is a higher percentage of absorbance in gembus tempeh than the soybean tempeh. In line, the result of t-test shows that there is a significant difference in the percent value absorbance of gembus tempeh compared to soybean tempeh (p=0.01). The mean percent absorbance of gembus tempeh (32.521%) is higher than soybean tempeh (17.016%). This case outlines that the antioxidant activity of gembus tempeh is higher than soybean tempeh. A similar trend was observed with Zhu's research, which reports that the high antioxidant activity of gembus Tempeh was related to the presence of fiber and peptides that exist in gembus tempeh. After 24 hours fermentation, the level of the peptide in gembus Tempeh was higher than soybean tempeh [29]. The other study had been found substances that contain antioxidants that were enhanced by the addition of in vivo lipid peroxidase [30].

The limitation of this study is an imbalance in the total number of study samples in tempeh wrappers; this is due to the availability of tempeh with PBBL wrappers which are rarely available in the traditional market. Its effect on the result data on material packaging of tempeh have not been able to conclude that a combination of polyethylene bag and banana leaf (PBBL) is better than polyethylene bag (PB). In addition, this study is cross sectional design where the researchers only observe objects in 1 time period.
4. CONCLUSION

This study shows that soybean and gembus tempeh have different antioxidant activity value; antioxidant activity in gembus tempeh is higher than soybean tempeh. The packaging of tempeh has no effect on antioxidant activity, it affects to the shelf life.

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REFERENCES

[1] Shurtleff W, Aoyagi A., “History of Tempeh and Tempeh Products, A Special Report on The History of Traditional Fermented Soyfoods,” Soyinfo Center, pp. 1-85, 2011.
[2] Astawan Made, Tuti W, Lulu M., “Tempeh,” 1st Ed. M ND, editor. Bogor: Publisher IPB Press, p. 197, 2017.
[3] Standard National Ins. Tempeh: Indonesian Tribute to Indonesia. Standard National Ins., pp. 1-10, 2012. [Online]. Available: www.BSN.go.id.
[4] IdoLimando, Bambang Martuliono Soewito, S.Sn., M.Sn, Adiel Yuwono SS., “Visual Book Design of Tempeh As One of Indonesian food.,” DRV Adiwarna, vol. 1, no. 4, pp. 1-12, 2014.
[5] Sarwono B., “Business of Tempeh and Oncom;” Penebar Swadaya, Jakarta, 2010.
[6] Suprapti ML., “Production of Tempeh,” Yogakarta, Kanistus, 2003.
[7] Gandjar I., Dewi I., Slamet S., Gizi P., Semboja U., Kesehatan D., et al., “Tempeh gembus as Fermented Tofu Dregs,” J. Penelit Gizi dan Makanan, vol. 2, pp. 70-9, 1972.
[8] Made A., Tutik W., Harmina BS., Nadia I., “Physicochemical Characteristics and Functional Properties of Tempeh Produced from Various Soybean,” Journal Pangan, vol. 22, no. 3, pp. 214-52, 2013, doi: 10.33964/jp.v22i3.102.
[9] Nazaretha R., Damanik S., Yanti D.Pratii Wi., et al., “Nutritional Composition Changes During Tempeh Gembus Processing Nutritional Composition Changes During Tempeh Gembus Processing,” 3rd International Conference on Tropical and Coastal Region Eco Development 2017, pp. 2-4, October 2018.
[10] Afifah DN, Nugrahani G., Hastuti VN, Arifan F., “The characteristics of Kerupuk Gembus,” IOP Conference Series Earth Environmental Science, vol. 292, 2019, doi:10.1088/1755-1315/292/1/012055.
[11] Agustina RK, Dieny FF, Rustanti N., Anjani G., Afifah DN., “Antioxidant activity and soluble protein content of tempeh gembushydrolyzate,” Hiroshima Journal Medical Sciences, vol. 67, Special issue, pp. 1-7, 2018.
[12] Afifah DN, Sulchan M., Syah D., Yanti, Suhartono MT., “Isolation and identification of fibrinolytic protease-producing microorganisms from Red Oncom and Gembus, Indonesian fermented soybean cakes,” Malaysia Journal Microbiology, vol. 10, no. 4, pp. 273-9, 2014.
[13] Noviana A., Dieny FF, Rustanti N., Anjani G., Afifah DN., “Antimicrobial activity of tempeh gembus hydrolyzate,” IOP Conference Series Earth Environmental Science., vol. 116, no. 1, 2018, pp. 1-8, doi: 10.1088/1755-1315/116/1/012044.
[14] Purwoko T., “concentration of isoflavone aglucone of soybean fermented with rhizopus microsporus var.oligosporus (tempeh): effect of soaking,” BioSMART, vol. 6, pp. 85–7, 2004.
[15] Chang CT, Hsu CK, Chou ST, Chen YC, Huang FS, Chung YC., “Effect of fermentation time on the antioxidant activities of tempeh prepared from fermented soybean using Rhizopusoligosporus,” International Food Sciences Technology, vol. 44, no. 4, pp. 799-806, 2009.
[16] Nurrahman, Astuti M., Suparome. Soesatyo MH., “The Mold Growth, Organoleptic Properties and Antioxidant Activities of Black Soybean Tempeh Fermented by different inoculums,” Agritech, vol. 32, no. 1, pp. 60-5, 2012.
Antioxidant activity of soybean and gembus tempeh (Sunarti)

[17] Murti B., “Epidemiological Research Principles and Methods,” UGM Press, 2013.
[18] Sastroasmoro SII., “Fundamentals of Clinical Research Methodology,” Sagung Seto, Jakarta, 2011.
[19] Dahlan MS., “Sample Size and Method of Sampling in Medical and Health Research,” Salemba Medika, Jakarta 2013.
[20] Andriani M., Ananditho BK, Nurhartadi E., “Effect of Drying Tempehature on physical and sensory characteristic of overripe tempeh float/Pengaruh suhu pengeringan terhadap karakteristik fisik dan sensorik tepung tempeh “bosok”,” Journal Teknologi hasil pertanian, vol. 6 no. 2, 2013.
[21] Mambang DEP, Suryanto D., “Antibacterial Activity of Tempeh Extracts on Bacillus subtilis and Staphylococcus aureus/Aktivitas bakteri ekstrak tempeh terhadap bakteri Bacillus subsitlis dan Staphilococcus aureus,” Jurnal Teknologi dan Pangan Industri, vol. 25, no 1, pp 115-8, 2014.
[22] Sovia E., Sukandar EY, Sasongko LDN, Sigit JI, Jenderal U., Yani A., et al., “Inhibition Activity of Garlic Extract and S-methyl Cysteine against the Reaction of the In Vitro Albumin Glication,” Jurnal Kedokteran Maranatha, vol. 10, no. 2, pp. 98-109, 2011.
[23] Kedare SB, Singh RP., “Genesis and development of DPPH method of antioxidant assay,” Journal Food Sciences Technology, vol. 48, no. 4, pp. 412-22, 2011.
[24] Sidiq M., Mappiratu M., Nurhaeni N., Study of Phenolic and Antioxidant Activity of Tempeh Extract from Various Incubation Times/Kajian kandungan fenolot dan aktivitas antioksidan ekstrak etanol tempeh gembus dari berbagai waktu inkubasi,” Jurnal Kovalen, vol. 2, no. 3, pp. 1-9, 2018.
[25] Hashim N, Tai CW, Wen HX, Ismail A, Kong KW., “Comparative Evaluation of Antioxidant Properties and Isoflavones of Tempeh Fermented in Two Different Wrapping Materials,” Current Research in Nutrition and Food Science Journal, vol. 6, no. 2, pp. 307-317, 2018, doi: 10.12944/CRNFSJ.6.2.06.
[26] Santhirasegaram V., George DS, Anthony KK, Singh HKB, Saruan NM, Razali Z, et al., “Effects of Soybean Processing and Packaging on the Quality of Commonly Consumed Local Delicacy Tempeh,” J Food Qual., vol. 39, no. 6, pp. 675-84, 2016.
[27] Widoyo S., “Effect of Time Fermentation on crude Fiber and Antioxidant Activities of Some Varieties of Soybean Glycine sp/Pengaruh lama fermentasi terhadap kadar serat kasar dan aktivitas antioksidan tempeh beberapa varitas kedelai,” Biofarmasi Journal of Natural Products Biochemistry, vol. 13 no 2.2015.
[28] Ariani SRD, Hastuti W., “Isoflavone Analysis and Antioxidant Activity Test on Tempeh with Variations in Fermentation Time and Extraction Method,” Pros Semin Nas Kim dan Pendidik Kim., vol. 5, pp. 568-80, 2009.
[29] Zhu YP, Fan JF, Cheng YQ, Li LT, “Improvement of the antioxidant activity of Chinese traditional fermented okara (Meitauza) using Bacillus subtilis B2,” Food Control, vol. 19, no. 7, pp. 654-61, 2008, doi: 10.1016/j.foodcont.2007.07.009.
[30] Matsuo M., “In Vivo Antioxidant Activity of Okara Koji, a Fermented Okara, by Aspergillusoryzae,” Biosci Biotechnol Biochemn., vol. 61, no. 12, pp. 1968-72, 1997, doi: 10.1271/bbb.61.1968.