International Journal of Agricultural Technology 2022Vol. 18(3):1339-1354
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

In vitro study of antioxidant and antimicrobial activities of soybean tempeh and split gill fungus (*Schizophyllum commune*) as plant-based diets

Yodsenee, K.[1,2], Suthirawut, S.[3], Pilasombut, K.[4,5], Urairong, H.[1] and Rumjuankiat, K.[1*]

[1]College of Agricultural Innovation and Food Technology, Rangsit University, Mueang Pathum Thani District, Pathum Thani, Thailand; [2]Faculty of Agricultural Technology, Rajamangala University of Technology Thunyaburi, Pathum Thani, Thailand; [3]Department of Microbiology, Faculty of Science, Kasetsart University, Bangkok, Thailand; [4]Department of Animal Production Technology and Fisheries, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand; [5]Excellence Center of Meat and Protein Innovation Technology, Thailand.

Yodsenee, K., Suthirawut, S., Pilasombut, K., Urairong, H. and Rumjuankiat, K. (2022). *In vitro* study of antioxidant and antimicrobial activities of soybean tempeh and split gill fungus (*Schizophyllum commune*) as plant-based diets. International Journal of Agricultural Technology 18(3):1339-1354.

Abstract Plant-based diets that reduce or eliminate animal product intake are known to ameliorate the incidence and clinical course of several illnesses. Soybean tempeh and split gill edible fungus (*Schizophyllum commune*) have long been consumed as plant-based diet choices. Total phenolic content (TPC), antioxidant and antimicrobial activities of soybean tempeh and *S. commune* extracts were investigated using water for extraction. The aqueous extract of fresh *S. commune* had the highest TPC at 1191.81 mg GAE/100g. Three methods were evaluated antioxidant activity as the diphenyl picrylhydrazyl radical scavenging test (DPPH), radical cation decolorization assay (ABTS) and reducing power. The IC_{50} values of fresh *S. commune* using DPPH and ABTS+ assays were 0.2812% and 0.5761%, respectively whereas the reducing power assay gave EC_{50} value at 0.3606%. Crude extracts of fresh tempeh and fresh *S. commune* exhibited antimicrobial activity against *Staphylococcus aureus* TISTR 746 at concentrations of 25 and 50 mg/ml, respectively. Two times the minimum inhibitory concentration (MIC) of fresh tempeh and fresh *S. commune* effectively reduced viable cells of *S. aureus* TISTR 746 of 5 log CFU/ml at 12 h. Both fresh tempeh and *S. commune* extracts showed promise as natural antioxidants for utilization as plant-based food.

Keywords: Plant-based diet, Antioxidant, Antimicrobial activity, *Schizophyllum commune*, Soybean tempeh

[*] **Corresponding Author:** Rumjuankiat, K.; **Email:** kittaporn.r@rsu.ac.th

Introduction

Increasing public awareness about healthy and sustainable foods, the environment, animal welfare and rejection of meat consumption on religious grounds has led to rising interest in plant-based diets (Corrin and Papadopoulos, 2017; Sabaté and Soret, 2014; Taufik *et al.*, 2019; Wild *et al.*, 2014), with increasing intake of fruits, vegetables, whole grains, legumes, nuts and seeds (Li, 2014; Taufik *et al.*, 2019). Plant-based diets have been shown to reduce the occurrence of type 2 diabetes and cardiovascular risk factors such as obesity, hypertension, hyperlipidemia, cardiovascular mortality, ischemic heart disease and cancer (Marsh *et al.*, 2011; McMacken and Shah, 2017; Tonstad *et al.*, 2009). Plant-based proteins have the potential to become major inexpensive and accessible commercial products (He *et al.*, 2020) as soy, mushroom, grain, natto, tofu and tempeh (Nils-Gerrit Wunsch, 2020).

Tempeh is a white, cake-formed product made by solid state fermentation of dehulled and boiled soybeans with added mold (Liu, 2008) such as *Rhizopus oligosporus, Rhizopus oryzae* and *Rhizopus stolonifer*. Tempeh is completely covered and penetrated by white mycelium of *Rhizopus* spp. and should not easily disintegrate when cut with a knife. Tempeh should have a clean smell that is devoid of ammonia with a meaty, mushroom-like nutty taste (Ahnan-Winarno *et al.*, 2021). Tempeh is nutritious and contains higher peptides, free amino acids and γ-aminobutyric acid than unfermented soybeans (Ito *et al.*, 2020). The product also has health-promoting antioxidant, antimicrobial, anticancer, antihypertensive, antithrombotic and hypocholesterolemic characteristics (Sanjukta and Rai, 2016). Tempeh is widely used as a plant-based protein in vegan and other types of contemporary meals such as burger patties, sausages, nuggets and in stews (Sihite *et al.*, 2018; Syamsuri *et al.*, 2020). Tempeh is also utilized as a meat extender, with low production cost and a high nutritional profile including fiber, vitamins and minerals (Ahnan-Winarno *et al.*, 2021).

Edible and medicinal mushrooms represent one of the world's greatest untapped resources of nutritious and healthy food, especially edible mushrooms once called the "food of the gods" (Dulay *et al.*, 2016). Among these, *Schizophyllum commune* Fr. (Family: Schizophyllaceae) is a widely distributed edible fungus that grows naturally on rotting wood. The genus *Schizophyllum* means "split gill" and *S. commune* is also known as the split gill fungus (Imtiaj *et al.*, 2008). It has a great prominence in the pharmaceutical and food industries because it produces metabolites that are essential in industrial products (Dulay *et al.*, 2016; Reyes *et al.*, 2009), especially schizophyllan, a polysaccharide derived from mycelia of *S. commune*, that has significant

antitumor and immunomodulating activities (Basso *et al.*, 2020). *S. commune* also contains various antioxidant compounds such as hydroxybenzoic acid, protocatechuic acid and tocopherol (Basso *et al.*, 2020; Liu *et al*., 2015). Crude extracts of *S. commune* can also inhibit several pathogens such as *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes* and *Klebsiella pneumonia* (Liu *et al.*, 2015). Therefore, this preliminary study investigated the antioxidant and antimicrobial activities of fresh/steamed tempeh and *S. commune* as possible future plant-based protein formulations.

Materials and methods

Tempeh production

Tempeh preparation followed the method of Gunawan-Puteri *et al.* (2015) with slight modifications. Commercial dehulled soybeans (*Glycine max*), 600 g were soaked in water for 24 hours at room temperature before washing and boiling in water for 30 min, draining and cooling to room temperature. After that, the soaking step was repeated and the soybean samples were drained, washed a few times and sterilized in hot water for 20 min. Finally, the commercial starter (Raprima Tempeh Starter, Indonesia) was inoculated with the ratio of soybean to starter at 1 kg:2 g. The inoculated beans were packed in perforated polyethylene bags and aerobically incubated at room temperature for 48 hours.

Cultivation of Schizophyllum commune's fruiting bodies

Wild macrofungi namely *Schizophyllum commune* or split gill edible fungus were collected from Southern Thailand**.** The mycelia of *S. commune* were isolated from the fruiting body by aseptically transferring an inner part of the tissue to a potato dextrose agar (PDA, Merck, Germany) plate before incubating at room temperature for 5 days to allow mycelial growth. The pure culture was punched using sterilized cork borer No.2, transferred to the mother spawn cultures of sterilized sorghum grains, and incubated at room temperature for 14 days. Thereafter, mycelia from the mother spawn cultures was transferred to commercial spawn bags containing rubber tree sawdust, rice bran, dolomite and water (100:50:1:75 kg) at 600 g/bag and incubated in the dark at room temperature until the mycelium was fully colonized on the substrate. Each spawn bag was then cut in four vertical stripes and a skew stripe and incubated for 7 days before harvesting. This method was modified from Garc á *et al.* (2018).

Preparation of crude extracts from soybean tempeh and Schizophyllum commune

Antioxidant activity of four samples consisting of fresh tempeh, steamed tempeh, fresh *S. commune* and steamed *S. commune* was measured. The steamed samples of tempeh and *S. commune* were prepared from fresh tempeh and *S. commune* by steaming with boiling water at 100 ℃ for 15 min. The samples were cut into small pieces and dried in a hot air oven (Binder, Germany) at 60 ℃ overnight. The extracts were prepared and modified following the method of Mirfat *et al.* (2014). Dried samples (approximately 50 g) were extracted with distilled water (ratio of 1:4), then mixed and soaked in a flask for 3 days. After maceration, the mixtures were centrifuged and filtrated through filter paper No.1 (Whatman, England). Finally, the filtrates were concentrated using a rotary evaporator (Buchi, Cannada) at low temperature (40 ℃) to obtain the crude extract and kept at -4 ℃ until required for use.

Determination of total phenolic content (TPC)

The *in vitro* total phenolics of the crude extracts were determined using the Folin-Ciocalteu assay as described by Cempaka *et al.* (2018). Briefly, 1 ml of each crude extract was mixed with 4.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu reagent (Sigma-Aldrich, USA). After 5 min, 4 ml of 7.5% sodium carbonate (KemAus, Australia) was added. The mixtures were allowed to stand for 60 min in the dark at room temperature. Each mixture was then centrifuged at 6,000 rpm for 5 min, and the absorbance was measured at 765 nm using a spectrophotometer (V-1200, Mapada, China). Finally, a calibration curve was plotted using gallic acid (Sigma-Aldrich, USA) as the standard reference, with results expressed as gallic acid equivalent per 100 g of sample (mg GAE/100 g of sample).

In vitro testing of DPPH radical scavenging activity

The 2,2-diphenyl-1-picrylhydrazyl-hydrate (DPPH) radical scavenging activity was evaluated according to the modified of Meng *et al*. (2016). Two milliliters of each crude extract were added to 2 ml of 0.1 mM DPPH (Sigma-Aldrich, USA) radical solution prepared in 95% ethanol. The mixtures were then incubated for 30 min in the dark at room temperature and measured at 517 nm using a spectrophotometer. Scavenging activity of the samples against the DPPH radical was expressed as percentage of inhibition according to the equation below:

$$\% \text{ Inhibition} = [\frac{(\text{A Control} - \text{A sample})}{\text{A Control}}] \times 100$$

where A_{sample} is the absorbance of 2 ml of extract mixed with 2 ml of 0.1 mM DPPH, and $A_{Control}$ is the absorbance of 2 ml of 95% ethanol mixed with 2 ml of 0.1 mM DPPH. Sample concentrations providing 50% inhibition (IC_{50}) were calculated from a graph plotted of percentage inhibition against sample concentration. Two chemicals including α-tocopherol (Sigma-Aldrich, USA) and 2, 6-di-tert-butyl-4-methylphenol (BHT, Acros, Belgium) were used as standards.

ABTS radical cation inhibition activity

The ABTS assay was modified from the method of Slima *et al.* (2018). The stock 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^+$) solution was generated by mixing two solutions of 7 mM $ABTS^+$ (Sigma-Aldrich, USA) and 2.4 mM potassium persulfate (Merck, Germany). The mixture was allowed to stand for 12-16 hours in the dark at room temperature. This solution was then diluted with 95% ethanol to display absorbance of 0.70±0.02 at 734 nm. Three milliliters of diluted $ABTS^+$ solution were added to 0.3 ml of each crude extract. The mixtures were then incubated for 6 min in the dark at room temperature, and absorbance was measured at 734 nm using a spectrophotometer. Scavenging activity of the samples against ABTS radical cation decolorization was expressed as percentage of inhibition and calculated as:

$$\% \text{ Inhibition} = [\frac{(\text{A Control} - \text{A sample})}{\text{A Control}}] \times 100$$

where A_{sample} is the absorbance of 0.3 ml of extract mixed with 3 ml of $ABTS^+$ solution, and $A_{Control}$ is the absorbance of 0.3 ml of 95% ethanol mixed with 3 ml of $ABTS^+$ solution. Sample concentrations providing 50% inhibition (IC_{50}) were calculated from a graph plotted of percentage inhibition against sample concentration. Two chemicals consisted of α-tocopherol (Sigma-Aldrich, USA) and 2, 6-di-tert-butyl-4-methylphenol (BHT, Acros, Belgium) were used as standards.

Reducing power

Reducing power ability was investigated following the method of Vijayalakshmi and Ruckmani (2016). In brief, 1 ml of each crude extract was transferred to 2.5 ml of 0.2 M phosphate buffer (pH 6.6), mixed with 2.5 ml of

1% potassium ferricyanide in a test tube and incubated at 50 ℃ for 30 min. The mixture was added with 2.5 ml of 10% trichloroacetic acid (TCA, Merck, Germany) and centrifuged at 3,000 rpm, 25°C for 10 min. Then, 2.5 ml of supernatant was collected and diluted with 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride (Unilab, New Zealand). The mixtures were incubated at room temperature for 30 min, and the absorbance was measured at 700 nm using a spectrophotometer. α-Tocopherol and BHT were used as standards. Values of half maximal effective concentrations (EC_{50}) were calculated from the graph plotted for absorbance at 700 nm.

In vitro testing of extracts for antimicrobial activity using agar well diffusion

Antimicrobial activities of various extracts were evaluated by the agar well diffusion method described by Srichanun *et al.* (2018). Antimicrobial activity was expressed as minimum inhibitory concentration (MIC). *Staphylococcus aureus* TISTR 746 was cultured as the indicator strain in tryptic soy broth (TSB, Merck, Germany) and incubated at 37 ℃ overnight. A number of *S. aureus* TISTR 746 cells were adjusted to 10^8 CFU/ml or 0.5 McFarland standard using 0.1% peptone water diluent. The bacterial inoculum was spread using a sterile cotton swab on a sterile petri tryptic soy agar (TSA, Merck, Germany) plate. After inoculation, the plates were dried for 10 min and the wells were aseptically punched using sterile cork borers No.2. Consequently, concentrations of various crude extracts were diluted as 100, 50, 25 and 12.5 mg/ml by two-fold serial dilutions using sterile distilled water. Each crude extract concentration was introduced into the well at about 50 µl, while sterile distilled water was performed as a control. The plates were incubated at 37 ℃ for 24 hours. This experiment was carried out in triplicate, and the diameter of the zone of inhibition was measured. Antimicrobial activity was determined by measuring the diameter of the inhibition zone (mm) with the lowest concentration and expressed as the minimum inhibitory concentration (MIC).

Time-kill kinetic assay

The time-kill kinetic of various extracts consisting of fresh tempeh and fresh *S. commune* was carried out following the method of Appiah *et al.* (2017). An overnight culture of *S. aureus* TISTR 746 was mixed with fresh tempeh and fresh *S. commune* crude extract. The final concentration of *S. aureus* TISTR 746 in the mixture was approximately 5 log CFU/mL, whereas final concentrations of the four crude extracts were performed at 1x, 2x and 4xMIC. Sterile distilled water without the extracts was used as a control. All the

mixtures were incubated at 37 ℃. After 0, 30, 60, 120, 180, 240, 360, 480 and 720 min of incubation, the bacterial viability was evaluated by serially diluting 100 µL of the mixture and spreading onto a tryptic soy agar plate.

Statistical analysis

Data were expressed as mean ± standard deviation. Significant differences between groups were determined by one-way analysis of variance (ANOVA) using software package IBM SPSS version 22. Duncan's Multiple Range Test was used to compare mean values at a significance level of $p< 0.05$.

Results

Total phenolic content (TPC) analysis

Phenolic compounds are the primary antioxidants in plant-based food, vegetables, fruits and grains. The TPC of the crude extracts was determined by the Folin-Ciocalteu assay, with results shown in Table 1. Highest TPC value of fresh *S. commune* was 1191.81±9.37 mg GAE/100 g, while TPC of crude extracts including steamed *S. commune*, fresh tempeh and steamed tempeh were 234.96±1.07, 244.45±24.2 and 55.03±12.8 mg GAE/100 g, respectively.

Table 1. Total phenolic content of the four crude extracts

Sample	**Total phenolic content** (mg GAE/ 100g sample)
Fresh tempeh	244.45 ± 24.2^{b}
Steamed tempeh	55.03 ± 12.8^{b}
Fresh *S. commune*	1191.81 ± 9.37^{a}
Steamed *S. commune*	234.96 ± 1.07^{b}

Values are reported as mean ± standard deviation. Different superscripts in the same column indicate significant differences ($p < 0.05$); measured by one-way ANOVA and Duncan's test.

Evaluation of antioxidant activity in crude extracts

Antioxidant activities of the extracts were assessed using different assays such as DPPH, ABTS and reducing power, with results shown in Table 1. Reduction capabilities of the DPPH radical and $ABTS^{+}$ were expressed as IC_{50} values, while reducing power was expressed as EC_{50} and considered as the efficiency to reduce Fe^{3+} to Fe^{2+}. Decreasing IC_{50} and EC_{50} values corresponded

to higher antioxidant activity. When comparing the IC_{50} and EC_{50} values, highest antioxidant activity was found in fresh *S. commune*. Highest IC_{50} values were 0.2812 and 0.5761, with high EC_{50} at 0.3606. The IC_{50} and EC_{50} values of the standards using synthetic antioxidants consisting of BHT and α-tocopherol were less than all four samples of fresh/steamed tempeh and fresh/steamed *S. commune*, as shown in Table 2.

Table 2. Values of antioxidant activity as free radical scavenging activity, radical cation decolorization and reducing power of the four crude extracts from fresh tempeh, steamed tempeh, fresh *S. commune* and steamed *S. commune*

Treatment	IC_{50} (%)		EC_{50} (%)
	DPPH	$ABTS^+$	Reducing power
Sample			
Fresh tempeh	$17.2704 \pm 1.08^{c1/}$	1.7754 ± 0.41^{c}	12.6249 ± 0.41^{c}
Steamed tempeh	21.2021 ± 1.37^{d}	29.9171 ± 2.68^{e}	21.4146 ± 0.28^{e}
Fresh *S. commune*	0.2812 ± 0.00^{b}	0.5761 ± 0.12^{b}	0.3606 ± 0.02^{b}
Steamed *S. commune*	22.2765 ± 0.46^{e}	2.2339 ± 0.55^{d}	7.6025 ± 0.04^{d}
Standard			
BHT	0.0021 ± 0.00^{a}	0.0022 ± 0.00^{a}	0.0054 ± 0.00^{a}
α-tocopherol	0.0002 ± 0.00^{a}	0.0014 ± 0.00^{a}	0.0059 ± 0.00^{a}

Values are reported as mean ± standard deviation. Different superscripts in the same column indicate significant differences ($p < 0.05$).

Table 3. Sensitivity testing of crude extracts against *S. aureus* TISIR 746 using the agar well diffusion method

Sample	Clear zone diameter (mm)			
	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml
Fresh tempeh	11.43 ±0.66	9.76 ±0.56	8.86 ±0.75	NA
Steamed tempeh	NA	NA	NA	NA
Fresh *S. commune*	10.23 ±0.32	8.36 ±0.50	NA	NA
Steamed *S. commune*	NA	NA	NA	NA

NA = No activity

Determination of antimicrobial activity of the four crude extracts

Inhibition activities of the four various crude extracts against *S. aureus* TISTR 746 as the indicator strain was determined. Crude extract samples of fresh tempeh and fresh *S. commune* inhibited this indicator strain, with lowest concentrations of 25 and 50 mg/ml, respectively and inhibition zone diameters of 8.86 and 8.36 mm. However, extracts of steamed tempeh and steamed *S. commune* were not effective against this indicator strain (Table 3).

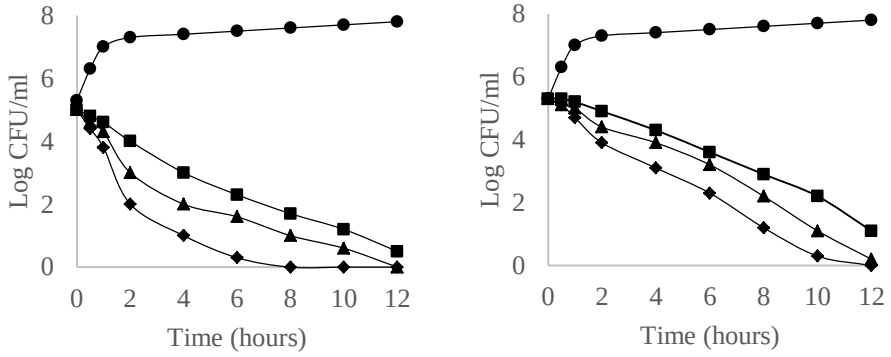

Figure 1. Time-kinetic profile of different concentrations of crude extracts of fresh tempeh (A) and fresh *S. commune* (B) exposed to *S. aureus* TISTR 476 for 12 hours. Concentrations of crude extract comprised 1xMIC (■), 2xMIC (▲) and 4xMIC (♦). Sterile distilled water was used as a control (●).

Killing time enumeration

The time-kill kinetic assay against *S. aureus* TISTR 476 was investigated using MIC values of fresh tempeh crude extract and fresh *S. commune*. After 12 hours incubation of *S. aureus* TISTR 476 containing 1xMIC, 2xMIC and 4xMIC crude extracts, results demonstrated that viable cells of *S. aureus* TISTR 476 gradually reduced from 5 log CFU/ml. Results of the time-kill assay are shown in Figure 1. The time-kill kinetic profile of fresh tempeh extract at 4xMIC (100 mg/ml) and 2xMIC (50 mg/ml) displayed that *S. aureus* was completely killed when exposed to crude extract at 8 hours and 12 hours, respectively. Concentrations of fresh *S. commune* extract at 4xMIC and 2xMIC also completely killed the indicator strain at 12 hours. However, viable cells of *S. aureus* slightly decreased from approximately 5 log CFU/ml to 1 log CFU/ml when using 1xMIC (25 mg/ml) for both crude extracts. The survival rate of *S. aureus* TISTR 746 without crude extracts remained constant at approximately 7 log CFU/ml until 12 hours.

However, viable cells of the *S. aureus* strain slightly decreased from approximately 5 log CFU/ml to 1 log CFU/ml using 1xMIC (25 mg/ml) for both crude extracts, while the survival rate of *S. aureus* TISTR 746 without crude extracts remained constant at approximately 7 log CFU/ml until 12 hours, as shown in Figure 1.

Discussion

Plant-based foods are well known to be high in nutrients such as carbohydrates, lipids, fiber, minerals and essential vitamins (Hever and Cronise, 2017). Recently, plants have attracted considerable attention as antioxidants (Amarowicz and Pegg, 2019), with potential roles in ameliorating diseases (Tran *et al.*, 2020; Johannesen *et al.*, 2020; Molina-Montes *et al.*, 2020; Jabri *et al.*, 2021). Numerous studies have confirmed the benefits of plant antioxidants as additives to improve the nutritional value of food (Lourenço *et al.*, 2019). Synthetic antioxidants are normally used in food preservation but recently increased public attention has promoted interest in natural healthy alternatives to replace synthetic products containing toxicological and carcinogenic agents (Xu *et al.*, 2021; Shahidi and Ambigaipalan, 2015). Many food ingredients are consumed as fresh or cooked using various processes such as stewing, stir-frying, or steaming (Hwang *et al.*, 2012). Here, fresh and cooked samples soybean tempeh, commonly served in Indonesian daily meals (Kuligowski *et al.*, 2017; Mani and Ming, 2017) and split gill mushroom (*S. commune*) found in Southern Thailand (Puket *et al.*, 2019) were investigated for their antioxidant activity. Selection of an appropriate extraction procedure depends on the plant, solvent used (water, ethanol, methanol, acetone and hexane), pH of the solvent, temperature and solvent to sample ratio (Sardarodiyan and Mohamadi Sani, 2016; Hidalgo and Almajano, 2017; Abu *et al.*, 2017). Kobus-Cisowska *et al.* (2020) found that organic solvents including acetone and ethanol were appropriate to extract plant samples. However, water is widely used as a natural solvent for antioxidant extraction because it is cheap, nontoxic and nonflammable (Charoenteeraboon *et al.*, 2010). Pandey and Rizvi (2009) reported the advantages of phenolic compounds including anti-aging, anti-inflammatory and the treatment of various diseases such as cancer and diabetes. In this study, highest TPC was obtained from fresh *S. commune* extract at 1191.81 mg GAE/100g sample (Table 1), while other studies recorded 6-16 mg GAE/g (Abdullah *et al.*, 2012; Yim *et al.*, 2013) Moreover, the TPC content of tempeh (2.44 mg GAE/g) in our study concurred with Hashim *et al.* (2018) who reported 1.74-6.97 mg GAE/g.

Many mushrooms are rich in carbohydrates (β-glucans), phenolic compounds (tocopherols), B-vitamins (niacin, flavin and pyridoxine), organic acids (ascorbate, shikimate, malate and fumarate), monoterpenoids and diterpenoids, lipids and proteins. Mushrooms have long been part of the human diet as valuable health foods (Khatua *et al.*, 2013). Edible mushrooms contain antioxidant compounds such as hydroxybenzoic acid, protocatechuic acid, beta-glucan flavonoid and tocopherol (Ferreira *et al.*, 2009; Özcan and Ertan *et al.*, 2018; Shaffique *et al.*, 2021) that protect against oxidative damage in the

human body. *S. commune* has also shown medical benefits from its contained biologically active compounds, with high potential to promote health (Mayakrishnan *et al.*, 2013). In our study, steamed *S. commune* had lower TPC content than fresh *S. commune* as a result of loss during the cooking process. Ranilla *et al*. (2009) investigated how different cooking conditions impacted the antioxidants contained in Brazilian bean (*Phaseolus vulgaris* L.). Their results showed that cooking the beans either at 100 or 121 ℃ without a soaking stage and keeping the cooking water was optimal for retaining antioxidant phenolic compounds. Hwang *et al.* (2012) attributed reduced antioxidant compounds in cooked foods to dissolution in the cooking water, while Hwang (2019) found that total polyphenol and flavonoid contents of fresh cauliflower extract were higher than both steamed and boiled cauliflower extracts. Thermal processing and long-term cooking decreased antioxidant capability and vitamin content according to the literature (Lešková *et al.*, 2006; Grajek and Olejnik, 2010).

Emsen *et al.* (2017) reported that phenolic compounds or antioxidants found in numerous plant species showed defensive mechanisms against attack by pathogenic bacteria. Plant extract mechanisms against antimicrobial activity also depend on the types of biologically active compounds and bacterial species (Farhadi *et al.*, 2019; Matilla-Cuenca *et al.*, 2020). Important mechanisms of antioxidant antimicrobial action include interaction with the bacterial cell wall or inhibition of cell wall formation (Mickymaray *et al.*, 2020). Plant extracts can destroy the cell wall of both gram-positive and gram-negative bacteria (Angiolella *et al.*, 2018). Several reports detailed the antimicrobial inhibition of legume extracts. Extract of tempeh inhibited *Bacillus subtilis*, *Bifidobacterium*, *Escherichia coli* (Kuligowski *et al*., 2013), *Streptococcus mutans* and *B. subtilis* (Noviana *et al.*, 2017) and *Actinomyces viscosus* (Sathiaseelan *et al.*, 2017). Appiah *et al.* (2017) found that the antioxidant and antimicrobial activities of *S. commune* extract inhibited pathogens including *Candida albican*, *Aspergillus niger*, *Klebsiella pneumonia*, *E. coli*, *B. subtilis* and *S. aureus.*

In this study, extracts of soybean tempeh and *S. commune* inhibited *S. aureus* TISTR 746 (Table 3), showing clear zone inhibition of raw treatments, while steamed treatments did not inhibit this indicator strain because inhibitory capability reduced on exposure to heating (Siritrakulsak and Simla, 2015). The time-kill results of fresh tempeh and fresh *S. commune* extracts against *S. aureus* TISTR 746 are shown in Figure 1. Concentrations of fresh tempeh extract at 4xMIC (100 mg/ml) and 2xMIC (50 mg/ml) completely killed viable cells of *S. aureus* when exposed to crude extract at 8 and 12 hours, whereas fresh *S. commune* extract at 4xMIC and 2xMIC killed the indicator strain

completely at 12 hours. Appiah *et al*. (2017) reported killing time of *S. commune* extracted by methanol at a concentration of 6 mg/ml against *E. coli*, *S. aureus* and *C. albicans* with decreasing numbers of viable cells over 6, 4 and 36 hours, respectively.

It concluded *in vitro* study of antioxidant activity of fresh/steamed tempeh and *S. commune* determined DPPH, ABTS and reducing power assay. The IC_{50} and EC_{50} values of fresh tempeh and fresh *S. commune* exhibited higher activity than the steamed treatments. Crude extracts of fresh tempeh and *S. commune* also inhibited foodborne bacteria. Therefore, soybean tempeh and *S. commune* showed potential as sources of natural antioxidants, with important future roles in food safety.

Acknowledgements

We express our gratitude to the College of Agricultural Innovation and Food Technology of Rangsit University and the Excellence Center of Meat and Protein Innovation Technology of King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand, for their support.

References

Abdullah, N., Ismail, S. M., Aminudin, N., Shuib, A. S. and Lau, B. F. (2012). Evaluation of selected culinary-medicinal mushrooms for antioxidant and ace inhibitory activities. Evidence-Based Complementary and Alternative Medicine, 464238.

Abu, F., Mat Taib, C. N., Mohd Moklas, M. A. and Mohd Akhir, S. (2017). Antioxidant properties of crude extract, partition extract, and fermented medium of *Dendrobium sabin* flower. Evidence-Based Complementary and Alternative Medicine, 2907219.

Ahnan-Winarno, A. D., Cordeiro, L., Winarno, F. G., Gibbons, J. and H. Xiao. (2021). Tempeh: A semicentennial review on its health benefits, fermentation, safety, processing, sustainability, and affordability. Comprehensive Reviews in Food Science and Food Safety, 20:1717-1767.

Amarowicz, R. and Pegg, R. B. (2019). Natural antioxidants of plant origin. Advances in Food and Nutrition Research, 90:1-81.

Angiolella, L., Sacchetti, G. and Efferth, T. (2018). Antimicrobial and antioxidant activities of natural compounds. Evidence-Based Complementary and Alternative Medicine, 1945179.

Appiah, T., Boakye, Y. D. and Agyare, C. (2017). Antimicrobial activities and time-kill kinetics of extracts of selected ghanaian mushrooms. Evidence-based complementary and alternative medicine, 4534350:1-15.

Basso, V., Schiavenin, C., Mendonça, S., de Siqueira, F. G., Salvador, M. and Camassola, M. (2020). Chemical features and antioxidant profile by *Schizophyllum commune* produced on different agroindustrial wastes and byproducts of biodiesel production. Food Chemistry, 329:127089.

Cempaka, L., Eliza, N., Ardiansyah, D. Handoko, D. and Astuti, R. M. (2018). Proximate composition total phenolic content and sensory analysis of rice bran tempeh. Makara Journal of Science, 22:89-94.

Charoenteeraboon, J., Ngamkitidechaku, C., Soonthornchareonnon, N., Jaijoy, K. and Sireeratawong, S. (2010). Antioxidant activities of the standardized water extract from fruit of *Phyllanthus emblica* Linn. Songklanakarin Jounal Science and Technology, 32:599-604.

Corrin, T. and Papadopoulos, A. (2017). Understanding the attitudes and perceptions of vegetarian and plant-based diets to shape future health promotion programs. Appetite, 109:40-47.

Dulay, R. M. R., Vicente, J. J. A., Cruz, A. D., Gagarin, J. M., Fernando, W., Kalaw, S. P. and Reyes, R. G. (2016). Antioxidant activity and total phenolic content of Volvariella volvacea and *Schizophyllum commune* mycelia cultured in indigenous liquid media. Mycosphere, 7:131-138.

Emsen, B., Kocabas, A., Kaya, A., Cinar, S., Aasim, M. and Said, G. (2017). *In vitro* cytotoxicity, antibacterial and antioxidant properties of various extracts from *Schizophyllum Commune* Fr. Fresenius Environmental Bulletin, 26:1144-1153.

Farhadi, F., Khameneh, B., Iranshahi, M. and Iranshahy, M. (2019). Antibacterial activity of flavonoids and their structure–activity relationship: An update review. Phytotherapy Research, 33:13-40.

Ferreira, I. C., Barros, L. and Abreu, R. M. (2009). Antioxidants in wild mushrooms. Current Medicinal Chemistry, 16:1543-1560.

Garc á, S. C., Carre ño Ruiz, S. D. and Hern ández, R. G. (2018). Fruit body production of *Schizophyllum commune*. In book: Updates on Tropical Mushrooms, 95-104.

Grajek and Olejnik (2010). The Influence of Food Processing and Home Cooking on the Antioxidant Stability in Foods. Functional Food Product Development (Smith, J., and Charter, E. /Functional Food Product Development), 178-205.

Gunawan-Puteri, M. D. P. T., Hassanein, T. R., Prabawati, E. K., Wijaya, C. H. and Mutukumira, A. N. (2015). Sensory characteristics of seasoning powders from overripe tempeh, a solid-state fermented soybean. Procedia Chemistry, 14:263-269.

Hashim, N., Woon Tai, C. W. T., Xin Wen, H. X. W., Ismail, A. and Kong, K. W. (2018). Comparative evaluation of antioxidant properties and isoflavones of tempeh fermented in two different wrapping materials. Current Research in Nutrition and Food Science Journal, 6:307-317.

He, J., Evans, N. M., Liu, H. and Shao, S. (2020). A review of research on plant-based meat alternatives: Driving forces, history, manufacturing, and consumer attitudes. Comprehensive Reviews in Food Science and Food Safety, 19:2639-2656.

Hever, J. and Cronise, R. J. (2017). Plant-based nutrition for healthcare professionals: implementing diet as a primary modality in the prevention and treatment of chronic disease. Journal of Geriatric Cardiology, 14:355-368.

Hidalgo, G. I. and Almajano, M. P. (2017). Red fruits: extraction of antioxidants, phenolic content, and radical scavenging determination: a review. Antioxidants (Basel), 6(1).

Hwang, I. G., Shin, Y. J. Lee, S. Lee, J. and Yoo, S. M. (2012). Effects of different cooking methods on the antioxidant properties of red pepper (*Capsicum annuum* L.). Preventive Nutrition and Food Science, 17:286-292.

Hwang, E. S. (2019). Effect of cooking method on antioxidant compound contents in cauliflower. Preventive Nutrition and Food Science Journal, 24:210-216.

Imtiaj, A., Jayasinghe, C., Lee, G. W., Kim, H. Y., Shim, M. J., Rho, H. S., Lee, H. S., Hur, H., Lee, M. W., Lee, U. Y. and Lee, T. S. (2008). Physicochemical requirement for the vegetative growth of *Schizophyllum commune* collected from different ecological origins. Mycobiology, 36:34-39.

Ito, M., Ito, T., Aoki, H., Nishioka, K., Shiokawa, T., Tada, H., Takeuchi, Y., Takeyasu, N., Yamamoto, T. and Takashiba, S. (2020). Isolation and identification of the antimicrobial substance included in tempeh using *Rhizopus stolonifer* NBRC 30816 for fermentation. International Journal of Food Microbiology, 325:108645.

Jabri, A., Kumar, A., Verghese, E., Alameh, A., Kumar, A., Khan, M. S. and Kalra, A. (2021). Meta-analysis of effect of vegetarian diet on ischemic heart disease and all-cause mortality. American Journal of Preventive Cardiology, 7:100182.

Johannesen, C. O., Dale, H. F., Jensen, C. and Lied, G. A. (2020). Effects of plant-based diets on outcomes related to glucose metabolism: a systematic review. Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy, 13:2811-2822.

Khatua, S., Paul, S. and Acharya, K. (2013). Mushroom as the potential source of new generation of antioxidant: a review. Research Journal of Pharmacy and Technology, 6:496-505.

Kobus-Cisowska, J., Szczepaniak, O., Szymanowska-Powałowska, D., Piechocka, J., Szulc, P. and Dziedziński, M. (2020). Antioxidant potential of various solvent extract from *Morus alba* fruits and its major polyphenols composition. Ciência Rural, Santa Maria, 50: e20190371.

Kuligowski, M., Pawłowska, K., Jasińska-Kuligowska, I. and Nowak, J. (2013). Evaluation of bean and soy tempeh infuence on intestinal bacteria and estimation of antibacterial properties of bean tempeh. Polish Journal of Microbiology, 62:189-194.

Kuligowski, M., Pawłowska, K., Jasińska-Kuligowska, I. and Nowak, J. (2017). Isoflavone composition, polyphenols content and antioxidative activity of soybean seeds during tempeh fermentation. Journal of Food, 15:27-33.

Lešková, E., Kubíková, J., Kováciková, E., Košická, M., Porubská, J. and Holcíková, K. (2006). Vitamin losses: Retention during heat treatment and continual changes expressed by mathematical models. Journal of Food Composition and Analysis, 19:252-276.

Li, D. (2014). Effect of the vegetarian diet on non-communicable diseases. Journal of the Science of Food and Agriculture, 94:169-173.

Liu, K. (2008). Food use of whole soybeans "soybeans: chemistry, production, processing, and utilization. Johnson, L. A., White, P. J. and Galloway, R. eds. AOCS Press, Urbana, IL, 441-481.

Liu, X., Frydenvang, K., Liu, H., Zhai, L., Chen, M., Olsen, C. E. and Christensen, S. B. (2015). Iminolactones from *Schizophyllum commune*. Journal of Natural Products, 78:1165-1168.

Lourenço, S. C., Moldão-Martins, M. and Alves, A. D. (2019). Antioxidants of natural plant origins: from sources to food industry applications. Molecules, 24:4132.

Mani, V. and Ming, L. C. (2017). Tempeh and other fermented soybean products rich in isoflavones, 453-474.

Marsh, K., Zeuschner, C. and Saunders, A. (2011). Health Implications of a vegetarian diet: a review. American Journal of Lifestyle Medicine, 6:250-267.

Matilla-Cuenca, L., Gil, C., Cuesta, S., Rapún-Araiz, B., Žiemytė, M., Mira, A., Lasa, I. and Valle, J. (2020). Antibiofilm activity of flavonoids on staphylococcal biofilms through targeting BAP amyloids. Scientific Reports, 10:18968.

Mayakrishnan, V., Abdullah, N., Abidin, M. H. Z., Fadzil, N. H. M., Johari, N. M. K., Aminudin N., and Abidin, N. Z. (2013). Investigation of the antioxidative potential of various solvent fractions from fruiting bodies of *Schizophyllum commune* (Fr.) mushrooms and characterization of phytoconstituents. Journal of Agricultural Science, 5:58-68.

McMacken, M. and Shah., S. (2017). A plant-based diet for the prevention and treatment of type 2 diabetes. Journal of Geriatric Cardiology, 14:342-354.

Meng, G. E., Tian, Y. C., Yang, Y. and Shi, J. (2016). Evaluation of DPPH free radical scavenging activity of various extracts of ligularia fischeri *in vitro*: a case study of shaanxi region. The Indian Journal of Pharmaceutical Sciences, 78:436-442.

Mickymaray, S., Alfaiz, F. A. and Paramasivam, A. (2020). Efficacy and mechanisms of flavonoids against the emerging opportunistic nontuberculous Mycobacteria. Antibiotics, 9:450.

Mirfat, A. H. S., Noorlidah, A. and Vikineswary, S. (2014). Antimicrobial activities of split gill mushroom *Schizophyllum commune* Fr. American Journal of Research Communication, 2:113-124.

Molina-Montes, E., Salamanca-Fernandez, E., Garcia-Villanova, B. and Sanchez, M. J. (2020). The impact of plant-based dietary patterns on cancer-related outcomes: a rapid review and meta-analysis. Nutrients, 12.

Noviana, A., Dieny, F. F., Rustanti1, N., Anjani, G., and Afifah, D. N. (2017). Antimicrobial activity of tempeh gembus hydrolysate. IOP Conference Series Earth and Environmental Science, 116:012044.

Nils-Gerrit Wunsch. (2020). Meat substitutes market in the U.S. - Statistics and Facts. Statista Dossier on The U.S. Meat Substitutes Market 62.

Özcan, Ö. and Ertan, F. (2018). Beta-glucan content, antioxidant, and antimicrobial activities of some edible mushroom species. Food Science and Technology, 6:47-55.

Pandey, K. B. and Rizvi, S. I. (2009). Plant polyphenols as dietary antioxidants in human health and disease. Oxidative Medicine and Cellular Longevity, 2:270-278.

Puket, S. R. N., Sangkaew, T., Chanapan, P. and Techoei, S. (2019). Biological activity of β-gulcans from edible mushroom, *Schizophyllum commune* in Thailand. International Journal of Applied Pharmaceutics, 11:110-112.

Ranilla, L. G., Genovese, M. I. and Lajolo, F. M. (2009). Effect of different cooking conditions on phenolic compounds and antioxidant capacity of some selected Brazilian bean (*Phaseolus vulgaris* L.) Cultivars. Journal of Agricultural and Food Chemistry, 57:5734-5742.

Reyes, R. G., Grassel, W. and Rau, U. (2009). Coconut water as a novel culture medium for the biotechnological production of *Schizophyllum*. Journal of Nature Studies, 7:1-6.

Sabaté, J. and Soret. S. (2014). Sustainability of plant-based diets: back to the future. The American Journal of Clinical Nutrition, 100:476S-482S.

Sanjukta, S. and Rai, A. K. (2016). Production of bioactive peptides during soybean fermentation and their potential health benefits. Trends in Food Science and Technology, 50:1-10.

Sardarodiyan, M. and Mohamadi Sani, A. (2016). Natural antioxidants: sources, extraction, and application in food systems. Nutrition and Food Science, 46:363-373.

Sathiaseelan, A., Shit, C. S. and Chai, T. T. (2018). Anti-biofilm activity of fermented soybean tempeh extracts and fractions against oral primary colonizer bacteria. International Conference on Drug Discovery and Development, 1-12.

Shaffique, S., Kang, S. M., Kim, A. Y., Imran, M., Aaqil Khan, M. and Lee, I. J. (2021). Current knowledge of medicinal mushrooms related to anti-oxidant properties. Sustainability, 13:7948.

Shahidi, F. and Ambigaipalan, P. (2015). Phenolics and polyphenolics in foods, beverages, and spices: Antioxidant activity and health effects – A review. Journal of Functional Foods, 18:820-897.

Sihite, N. W., Rusmarilin, H., Suryanto, D. and Sihombing, D. R. (2018). Utilization of jasmine flower extract as antimicrobial in tempeh sausage. IOP Conference Series: Earth and Environmental Science, 205:012037.

Siritrakulsak, P. and Simla, S. (2015). Effects of conventional cooking method on antioxidant content in moonflower. Khon Kaen Agriculture Journal, 43:875-880.

Slima, S. B., Ktari, N., Trabelsi, I., Moussa, H., Makni, I. and Salah, R. B. (2018). Purification characterization and antioxidant properties of a novel polysaccharide extracted from *Sorghum bicolor* (*L*.) seeds in sausage. International Journal of Biological Macromolecules, 106:168-178.

Srichanun, M., Lerssutthichawal, T., Nganwisuthiphan, T. and Chirapongsatonkul, N. (2018). Possibility of split mushroom *Schizophyllum commune* by product extracts as antimicrobial and antioxidant agent for aquaculture. International Journal of Agricultural Technology, 14:1969-1976.

Syamsuri, R., Dewayani, W., Septianti, E. and Maintang. (2020). Chemical characteristic and sensory of tempeh sausage on different soybean varieties and cooking methods variation. IOP Conference Series: Earth and Environmental Science, 575:012012.

Taufik, D., Verain, M. C. D., Bouwman, E. P., and Reinders, M. J. (2019). Determinants of real-life behavioural interventions to stimulate more plant-based and less animal-based diets: A systematic review. Trends in Food Science and Technology, 93:281-303.

Tonstad, S., Butler, T., Yan, R. and Fraser, G. E. (2009). Type of vegetarian diet, body weight, and prevalence of type 2 diabetes. Diabetes Care, 32:791.

Tran, E., Dale, H. F., Jensen, C. and Lied, G. A. (2020). Effects of plant-based diets on weight status: a systematic review. diabetes, metabolic syndrome, and obesity: Targets and Therapy, 13:3433-3448.

Vijayalakshmi, M. and Ruckmani, K. (2016). Ferric reducing antioxidant power assay in plant extract. Bangladesh Journal of Pharmacology, 11:570-572.

Wild, F., Czerny, M., Janssen, A. M., Kole, A., Zunabovic, M., and Domig, K. J. (2014). The evolution of a plant-based alternative to meat. Agro FOOD Industry Hi Tech, 25:45-49.

Xu, X., Liu, A., Hu, S., Ares, I., Martinez-Larranaga, M. R., Wang, X. and Martinez, M. A. (2021). Synthetic phenolic antioxidants: Metabolism, hazards, and mechanism of action. Food Chemistry, 353:129488.

Yim, H. S., Chye, F. Y., Rao, V., Low, J. Y., Matanjun, P., How, S. E., and Ho, C. W. (2013). Optimization of extraction time and temperature on antioxidant activity of *Schizophyllum commune* aqueous extract using response surface methodology. Journal of Food Science and Technology, 50:275-283.

(Received: 20 August 2021, accepted: 10 April 2022)