EJFA Emirates Journal of Food and Agriculture

Emirates Journal of Food and Agriculture 37: 1–11
doi: 10.3897/ejfa.2025.154102

RESEARCH PAPER

Effect of *Spirulina platensis* addition on physicochemical, bioactivity, and sensory properties of fermented soybean as a potential functional food product

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Academic editor: Maria Manuela Silva ♦ **Received** 27 March 2025 ♦ **Accepted** 16 September 2025 ♦ **Published** 20 October 2025

Abstract

In the last decade, fermented soybean called tempeh has gained popularity globally as a healthy food especially for vegan/vegetarian and organic food enthusiasts. In order to improve the functionality of tempeh, *Spirulina platensis*, which is known to have high antioxidant activity is added to tempeh. Due to the unpleasant odour of *Spirulina*, the supplementation level of *Spirulina* on tempeh needs to be optimized to obtain tempeh product which has good nutrition and functionality as well as acceptability by the consumers. In this study, *Spirulina* was added at 5–40 g/kg and the fermentation varied from 24 to 72 h. The nutritional values of *Spirulina* tempeh was then analyzed, including proximate composition as well as antioxidant, α-amylase inhibition, and angiotensin I-converting enzyme (ACE)-inhibition activity. To evaluate the influence of *Spirulina* addition on tempeh sensory, hedonic and intensity tests were performed. The result of the study showed that the best fermentation time was 48 h, no difference of nutrition was observed with different addition of *Spirulina*. However, the *Spirulina* addition on tempeh increased the antioxidant, α-amylase inhibition, and ACE-inhibition activities by 39–104.7%, 12.2–33.5% and 28.2–46.5% compared to tempeh without *Spirulina* addition. The highest antioxidant, α-amylase inhibition, and ACE-inhibition activities were obtained with addition of 40 g/kg, 40 g/kg, and 20 g/kg, respectively. The overall likeness was in the range of 5.07–6.98 from 1–9 scales, the value decreasing along with the increase of *Spirulina* addition. Based on the nutrition, bioactivity, and sensory test, addition of 20 g/kg of *Spirulina* is suggested. The result reveals that *Spirulina* tempeh is a potential functional food.

Keywords

Functional food, nutrition, sensory, *Spirulina*, tempeh

Introduction

Recently, there has been a raising concern about functional foods since the pandemic has increased public health awareness. Globally, the sales of fortified foods/functional food reaches up to $267 billion, in which the sales in US reaches $63 billion (Sloan 2020; Soocial 2024). China has the highest potential market of fortified food in Asia followed by Indonesia, Japan, Hong Kong, India, Vietnam, Saudi Arabia, Mexico, Malaysia, and Brazil (Mascaraque 2018; Sloan 2020). Functional food in Asia Pacific generated 14 billion USD in 2017 and predicted to reach 21,45 billion USD in 2023 (Wunsch 2020).

Tempeh has been gaining more popularity as a potential functional food worldwide. The global market of tempeh increased by 15.50% during the forecast period of 2022–2029 (DataBridge Market Research 2022). It is made from boiled soybean fermented with fungi *Rhizopus* sp. In its origin country, Indonesia, tempeh is the staple protein. Tempeh consumption in a week reach 0.139 kg/capita, which is higher than that of beef (0.009 kg/capita) and chicken (0.124 kg/capita) (BPS 2020). Tempeh has an affordable price with a meat-like flavor. In addition, tempeh contains several antioxidant compounds such as isoflavone, superoxide dismutase, and tocopherol (Syukri et al. 2022). Antioxidant compounds combat free radicals, thus preventing degenerative diseases such as atherosclerosis, coronary heart disease, diabetes mellitus, etc. (Phaniendra et al. 2015). There are several factors affecting the nutritional quality of tempeh, such as raw materials, type of inoculum, tempeh making process, degree of sanitation, fermentation conditions, and fermentation time (Mujianto 2013; Rizal et al. 2022).

In order to improve the functionality of tempeh, the addition of other compounds with high antioxidant content is beneficial. One of such compounds is *Spirulina*, a microalgae with a spiral form and high nutritional value and bioactive compounds such as phycocyanin (Moraes et al. 2011). Rahim et al. (2021) reported that *Spirulina* contains dry matter (77.54 g/100 g), protein (53.31 g/100 g), lipid (9.25 g/100 g), and carbohydrate (23.38 g/100 g). Moreover, *Spirulina* also contains some phytopigments with potent antioxidant, including chlorophyll a, chlorophyll b, carotenoids, C-phycocyanins, allophycocyanins, and phycoerythrin. Therefore, *Spirulina* is often added in food products such as yoghurt and ice cream to improve the antioxidant capacity of the product (Barkallah et al. 2017; Szmejda et al. 2018).

Nowadays, *Spirulina* is produced worldwide with a production of 3000 tonnes per year (Shimamatsu 2004). In a tropical country such as Indonesia, *Spirulina* has abundant availability due to the easy cultivation and suitable climate. It is usually cultivated in a beach and has a short cultivating period with higher productivity and protein content compared to other commodities. Although it has health benefits, its application on food products is still limited due to its undesirable sensory properties. Therefore, it can be added to food products at certain levels that can improve the health benefit of the product without changing the sensory properties. This study aimed to investigate the effect of *Spirulina* on physicochemical, functionality and sensory of tempeh as well as to determine the best *Spirulina* addition and fermentation time to obtain the highest functionality and sensory score of *Spirulina* tempeh. It also has been reported that prolonging the fermentation can enhance the production of bioactive compounds, which plays a role in the product's functionality (Starzyńska-Janiszewska et al. 2016). Therefore, this study investigated the potential functionality of *Spirulina* tempeh including antioxidant activity, antidiabetic activity, and antihypertension activity.

Material and methods

Material

Grobogan local non-GMO soybeans were purchased from Attempe, which is the local producer of organic tempeh in Prambanan, Special Region of Yogyakarta. The *Spirulina* powder was obtained from Attempe. The inoculum used was *Rhizopus oligosporus* obtained from Biotechnology Laboratory, Faculty of Agricultural Technology, Special Region of Yogyakarta. The enzyme used in this study included α-amylase enzyme (A3306), 1,1-Diphenyl-2-picryl-hydrazyl (DPPH), N-Hippuryl-His-Leu hydrate, Angiotensin Converting Enzyme from rabbit lung (A6778) were purchased from Sigma Aldrich. All of other reagents used in this research were all in analytical grade.

Spirulina tempeh production

Tempeh was made by washing the soybeans two times followed by soaking with a soybean-to-water ratio of 1:3 for 24 h. Subsequently, the soaked soybean was rinsed and boiled for 30 min, and then dehulled. The dehulled soybeans were soaked again for 24 h, then washed and steamed for 30 minutes. The next stage was draining the cooked soybeans and inoculation with 0.02% starter (mixed culture) followed by the addition of *Spirulina* powder (5, 10, 20 and 40 g/kg). The soybeans were wrapped in perforated plastic (2 cm distance between holes) and incubated at room temperature for 36 hours.

Proximate analysis

The water content in *Spirulina* tempeh was analyzed thermogravimetrically, the protein content was determined by the micro-Kjeldahl method, the fat content was determined by the Soxhlet method, the ash content was determined by thermogravimetry, and the carbohydrate content was calculated by difference (AOAC 2005).

Analysis of antioxidant activity

Antioxidants in *Spirulina* tempeh were extracted by the method described by Xu et al. (2007). Sample was prepared by grind them in mortar and pestle until fine paste. Sample was extracted in 80:20 (v/v) methanol:water and stirred by magnetic stirrer for 3 hours. Samples were then extracted by placing them in 18 hours in a dark room at room temperature. Samples were centrifuged afterwards at 3000 rpm at 4 °C for 10 minutes. The supernatants were collected and further analyzed. DPPH scavenging activity was assayed by the method described by Starzyńska-Janiszewska et al. (2016) with some modifications. As much as 0.2 mL of extracts were mixed in 2.8 mL DPPH solution (0.1 mmol/L in 80% methanol). The mixtures were

incubated in dark room for 30 minutes and the absorption was measured at 517 nm against 80% methanol as a blank. The antioxidant activity was calculated as follows.

$$\text{Antioxidant activity}(\%) = \frac{\text{Acontrol517} - \text{Aextract517}}{\text{Acontrol517}} \times 100\%$$

α-amylase inhibition assay

The inhibition of α-amylase was assayed by the method described by Apostolidis et al. (2007) with minor modification. As much as 500 µL of tempeh water extract (0.1 mg/mL) and 500 µL α-amylase (Sigma Aldrich) 2.0 U/mL in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) were incubated at 25 °C for 10 minutes. After pre-incubation, 500 µL of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) was added at timed interval and incubate at 25 °C for 10 minutes. The reaction was stopped by adding 1.0 mL of dinitrosalicylic acid color reagent, and the mixture was incubated in boiling water for 5 minutes. The test tubes were cooled to room temperature. The mixture was diluted in 10 mL distilled water. A blank control was prepared, using distilled water in place of the sample. The absorbance was measured at 540 nm and calculated as follows.

$$\alpha\text{-amylase inhibition}(\%) = \frac{\text{Acontrol540} - \text{Aextract540}}{\text{Acontrol540}} \times 100\%$$

Angiotensin I-converting enzyme (ACE)-inhibition assay

ACE inhibition activity was assayed by the method of Chaudhary et al. (2013) and Cushman and Cheung (1971) with a little modification. As much as 50 µL of sample (100 mg/L) with 50 µL of ACE at 25 mU/mL (Sigma Aldrich) was pre-incubated at 37 °C for 10 minutes. Subsequently, 150 µL of substrate hippuryl-L-histidyl- L-leucine (HHL) (Sigma Aldrich) in 5 mM in 50 mM potassium borate buffer pH 8.3 containing 0.5 M NaCl was added and incubated for 30 minutes at 37 °C. The reaction was terminated by 250 µL of 1.0 M HCl. The hipurric acid formed was then extracted by 1.5 mL of ethyl acetate by vortex mixing vigorously for 2 minutes. The solution was centrifuged at 4000 rpm for 5 minutes and 1.0 mL of ethyl acetate layer was transferred to clean test tube. The supernatant was heated at 100 °C in boiling water bath for 30 minutes. The hippuric acid was redissolved in 3.0 mL distilled water and the absorbance was measured at 228 nm using a UV-VIS spectrophotometer (Thermo Scientific Genesys 10). The ACE inhibition activity was calculated using the following formula:

$$\%ACE\text{inhibition} = \frac{A - B}{A - C} \times 100\%$$

where:
A = absorbance at 228 nm with ACE, without inhibitor
B = absorbance at 228 nm with ACE and inhibitor
C = absorbance at 228 nm without ACE and inhibitor

Sensory test

The samples were subjected to hedonic and subjective intensity of attributes including colour, colour intensity, saltiness, umami, bitterness, firmness and rancidity. The sensory test was conducted by 68 untrained panelists with 9 point scale for hedonic (1-dislike extremely to 9-extremely like) and subjective intensity (1-very low to 9-very intense). Tempeh was sliced to a 4×5×1 cm size, marinated in 7% NaCl brine solution for 5 min before deep fried at 170 °C for 3 min. The five samples were monodically presented in a white paper plate, coded with a three-digit random number, and served at room temperature to the panels. Samples were served in a balanced order to counteract first-order carry-over effects. Mineral water and cucumber were provided for rinsing palate between samples.

Statistical analysis

All of the data are presented as mean±SD (standard deviation), which were calculated by Microsoft Excel 2013. The data was analyzed using one-way analysis of variance (ANOVA) in IBM SPSS 25, followed by Duncan's Multiple Range Test (DMRT) for multiple comparison means ($p < 0.05$).

Results and discussion

Physicochemical properties of Spirulina tempeh

Effect of fermentation time

Rhizopus oligosporus produces several enzymes during fermentation thus causing composition changes during fermentation. Table 1 shows that the fat content decreased during fermentation, meanwhile the carbohydrate content increase during fermentation. A previous study by Rizal et al. (2022) obtained similar results that the fat content of tempeh decreased along with the length of fermentation. This could be due to the enzymatic activity which was able to hydrolize triglycerides into glycerol and free fatty acids to support the growth of *Rhizopus oligosporus* during fermentation. This current study also revealed that tempeh protein increased along with the length of fermentation and reached its maximum value at 60 h incubation (Table 1). During fermentation, *Rhizopus oligosporus* produces intracellular, extracellular and cellwall-bound proteases which degrades protein into free amino acids resulted in higher protein content (Heskamp and Barz 1998; Starzyńska-Janiszewska et al. 2015). It has been reported that approximately 25% of the initial protein was degraded, in which 65% becomes amino acids, 25% is assimilated into mold biomass, and the rest is oxidized (Sparringa and Owens 1999). The protein content obtained in this study (45.29–49.91 g/100 g) fulfil the requirement of Indonesian National Standard and higher than that of reported by Hidayah et al. (2012) (40.23–44.96 g/100 g).

Table 1. Nutritional value (g/100 g in dry weight) of tempeh with the addition of *Spirulina* at concentration 5 g/kg in five different fermentation times.

Fermentation time (h)	Moisture	Ash	Fat	Protein	Carbohydrate
24	61.82 ± 1.24^{a}	2.64 ± 0.01^{ab}	20.15± 0.24^{b}	46.33 ± 4.84^{ab}	30.88 ± 4.60^{a}
36	64.93 ± 0.85^{a}	2.64 ± 0.01^{ab}	14.70 ± 2.15^{a}	45.29 ± 1.92^{a}	37.38 ± 4.08^{b}
48	62.44 ± 3.31^{a}	2.69 ± 0.04^{bc}	17.74 ± 0.99^{a}	45.29 ± 5.17^{a}	34.28 ± 4.21^{ab}
60	63.17 ± 0.10^{a}	2.60 ± 0.03^{a}	13.43 ± 0.37^{a}	49.91 ± 3.75^{b}	34.05 ± 4.08^{ab}
72	64.38 ± 3.13^{a}	2.75 ± 0.02^{c}	13.18 ± 2.33^{a}	46.85 ± 3.03^{ab}	37.22 ± 0.68^{b}

$^{a-c}$Values with different superscript alphabet in one column are significantly different based on Duncan's test ($p < 0.05$).

Table 2. Nutritional value (g/100 g in dry weight) of tempeh with the addition of *Spirulina* in five different concentrations.

Addition of *Spirulina* (g/kg)	Moisture	Ash	Fat	Protein	Carbohydrate
0	59.03 ± 1.47^{a}	2.05 ± 0.11^{a}	21.14± 1.13^{a}	48.07 ± 1.31^{bc}	28.74 ± 1.13^{ab}
5	59.10 ± 0.47^{a}	1.98 ± 0.05^{ab}	19.45 ± 0.54^{a}	46.89 ± 2.28^{ab}	31.67 ± 0.54^{bc}
10	58.68 ± 0.55^{a}	2.10 ± 0.06^{a}	19.38 ± 0.42^{a}	45.03 ± 1.57^{a}	33.49 ± 0.42^{c}
20	58.57 ± 0.96^{a}	2.32 ± 0.09^{b}	20.34 ± 2.01^{a}	49.98 ± 1.30^{c}	27.36 ± 2.01^{a}
40	57.70 ± 1.51^{a}	2.41 ± 0.11^{b}	19.18 ± 0.22^{a}	47.85 ± 1.13^{bc}	30.57 ± 0.22^{abc}

$^{a-c}$Values with different superscript alphabet in one column are significantly different based on Duncan's test ($p < 0.05$).

Spirulina powder is known to contain 59–63% of protein which is considered a potential protein source (Grosshagauer et al. 2020). These results indicated that increasing the length of fermentation to a certain extent could improve the quality of the product, however if the fermentation is extended, it changed the sensory such as texture which become soft and release unpleasant odour (Rizal et al. 2022). Therefore, according to nutritional value and sensory, the recommended fermentation time is 48 h.

Effect of Spirulina *addition*

The effect of *Spirulina* addition on the nutritional value of tempeh is presented in Table 2. The results showed that in general, the addition of *Spirulina* did not significantly affect the moisture, ash, fat, protein, and carbohydrate content of tempeh. This may be attributable to the microbial activity during fermentation, which primarily utilize the nutrient compounds for supporting their growth. A slight increase in protein and carbohydrate were obtained at the addition of 20 and 10 g/kg *Spirulina*, respectively. The similar value of tempeh with or without addition of *Spirulina* could be explained by the low concentration of *Spirulina* added which might not cause a change in physicochemical composition of the tempeh. However, there is a significant increase in ash content by the addition of 20 and 40 g/kg *Spirulina*. Several previous studies demonstrated that the addition of *Spirulina* increase the ash content of the final products, such as yogurt, snack, and ice cream (Barkallah et al. 2017; Lucas et al. 2018; Szmejda et al. 2018). This could be due to the high amount of mineral in *Spirulina*, which is correlated with the ash content. Rahim et al. (2021) reported that *Spirulina* was rich in minerals, particularly potassium, phosphorus, calcium, magnesium and iron, having several essential roles in human body.

Functional property of *Spirulina* tempeh

Effect of fermentation time

The effect of fermentation times on antioxidant and antidiabetic activities is presented in Table 3. The antidiabetic activity was measured by α-amylase inhibition assay. The results showed that antioxidant activity increased along with the length of fermentation. A previous study by de Marco Castro et al. (2019) demonstrated that fermentation significantly increased the antioxidant activity of *Spirulina*. The antioxidant activity of fermented *Spirulina* achieved the maximum content at 24 h of fermentation and diminished afterwards. *Spirulina* contains phenolic compounds, γ-tocopherol, phycocyanin, β-carotene and xanthophyll, which are responsible for the antioxidant activity (Rodríguez De Marco et al. 2014). Moreover, prolonged fermentation time also caused an increase in antioxidant activity of tempeh. Several compounds, which are known to have potential antioxidant activity in tempeh, such as daidzein, genistein, and glycitein are usually formed during fermentation (Yoshari et al. 2023). However, increasing the fermentation time did not significantly affect the α-amylase inhibition in *Spirulina*

Table 3. Antioxidant and antidiabetic activities of tempeh with the addition of *Spirulina* at concentration 5 g/kg in five different fermentation times.

Fermentation time (h)	Antioxidant activities (%)	α-amylase inhibition (%)
24	23.93 ± 0.99^{a}	9.00 ± 0.23^{a}
36	33.97 ± 1.96^{b}	9.89 ± 0.83^{a}
48	43.93 ± 2.54^{c}	13.19 ± 1.05^{b}
60	53.45 ± 0.90^{d}	11.98 ± 0.88^{ab}
72	49.92 ± 1.48^{d}	10.49 ± 1.64^{ab}

*Values with different superscript alphabet in one column are significantly different based on Duncan's test ($p < 0.05$).

tempeh. Astawan et al. (2023) obtained a different result, reporting that over-fermented tempeh exhibits higher antidiabetic activity. Several compounds have been reported to have inhibitory effect on α-amylase such as phenolic compounds, peptides, nonstarch polysaccharides, and lipids (Gong et al. 2020). The results revealed that the best fermentation time for *Spirulina* tempeh based on the functionality is 60 h.

Effect of *Spirulina* addition

Antioxidant activity

The effect of *Spirulina* addition on antioxidant activity is presented in Figure 1. The results show that the antioxidant activity is rising along with the increasing concentration of *Spirulina* added on tempeh. The addition of *Spirulina* resulted in a 39–104.7% improvement of antioxidant activity. The highest improvement was achieved with the addition of 40 g/kg of *Spirulina* which increased the antioxidant activity by two times. These findings are in accordance with several previous studies (Barkallah et al. 2017; El-Anany et al. 2023; Şahin 2020; Szmejda et al. 2018), reporting that supplementation of *Spirulina* enhance the antioxidant activity in various food products. El-Anany et al. (2023) studied the effect of *Spirulina* addition (10–50 g/kg) on chicken mortadella and demonstrated similar results that the antioxidant activity of the chicken increased in response to the higher level of *Spirulina* addition. Recent studies have explored the antioxidant potential of microalgae, including *Spirulina*. This antioxidant properties primarily derived from their abundance in various free radical scavengers (Barkallah et al. 2017). Fithriani et al. (2015) reported that *Spirulina* has antioxidant activity with IC_{50} of 518.94 ppm. *Spirulina* contained several phytochemical compounds which are responsible to the antioxidant activities, including phenolic (7.00 mg GAEs/g), flavonoid (1.00 mg QEs/g), carotenoid, C-phycocyanins (18.25 mg/g), allophycocyanins (5.34 mg/g), and phycoerythrin (3.47 mg/g) (Rahim et al. 2021). Therefore, the addition of *Spirulina* could improve the antioxidant activity of tempeh. This improvement is higher than those reported in other food products, such as yogurt and ice cream supplemented with *Spirulina* (Barkallah et al. 2017; Szmejda et al. 2018). However, the mechanisms underlying the antioxidant activity of *Spirulina* are rarely discussed in earlier studies. Phycocyanin and β-carotene are considered the major contributors to the antioxidant activities of *Spirulina*. These compounds help to protect against oxidative stress by scavenging free radicals, inhibiting lipid peroxidation, and modulating several key signaling pathways (Wu et al. 2016).

α-amylase inhibition activities

Similar to antioxidant activity, the addition of *Spirulina* increased the α-amylase inhibition activity, which is related to its antidiabetic potential. The higher the *Spirulina* added, the higher α-amylase inhibition activity was observed (Figure 2). The highest α-amylase inhibition activity was obtained at 40 g/kg *Spirulina* with the value of 14.06%. This value is in the range of the reported studies, in which α-amylase inhibition activity of *Spirulina* varied from 5% to 96% depending on the addition level and extraction solvent used (Agustiar et al. 2022). Extraction of *Spirulina* leads to a higher α-amylase activity, since in this study the *Spirulina* was added as a whole biomass without extraction this might explain the lower α-amylase inhibition activity. However, the earlier studies mostly focused on the discussion about the antidiabetic activity of pure microalgae biomass. Moreover, the mechanism underlying the antidiabetic activity of *Spirulina* has not yet been completely understood, but several studies have discussed the potential mechanisms, such as enzyme inhibition, the prevention of oxidative stress, and the improvement of insulin sensitivity (El-Sakhawy et al. 2023; Vieira et al. 2021). *Spirulina* has been reported to have antioxidant compounds, such as phycocyanin and carotenoids, which can prevent the oxidative stress, which in turn may lower the risk of metabolic disorders, including diabetes (Kumar et al. 2022). Therefore, the increased α-amylase inhibition activity may be attributable to the antioxidant activity of *Spirulina* tempeh. The α-amylase inhibition activity also could be affected by the high amount of ω-6 PUFA in *Spirulina*, such as linoleic acid (144.81 mg/100 g) and γ-linolenic acid (1866.27 mg/100 g), which are responsible for antidiabetic activity (Guldas et al. 2021). Moreover, the

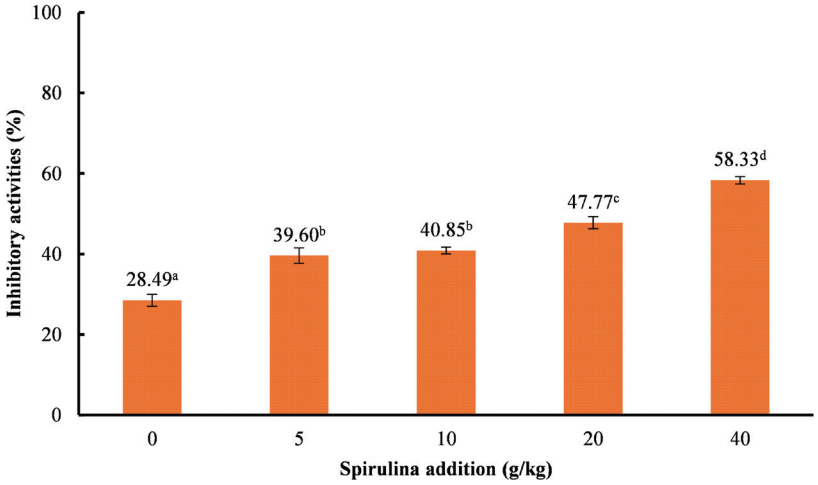

Figure 1. Antioxidant activities of tempeh with the addition of *Spirulina* in five different concentrations.

presence of bioactive peptides in *Spirulina* may contribute to the antidiabetic activity. Hu et al. (2019) identified several bioactive peptides (GVPMPNK, RNPFVFAPTLLTVAAR and LRSELAAWSR) from *Spirulina platensis*, which exhibit potent inhibition on α-amylase. Tempeh also contains peptides, insulinotropic amino acids, isoflavones, genistein and daidzein which have been reported to play a role in antidiabetic activity (Yoshari et al. 2023). Therefore, *Spirulina* addition on tempeh at the highest concentration could increase 33.52% of α-amylase inhibition activity.

ACE inhibition activity

The effect of *Spirulina* addition on ACE inhibitory activity of tempeh is presented in Figure 3. The result shows that the addition of *Spirulina* increased 28.2–46.5% of ACE inhibitory activity. The highest ACE inhibition activity was obtained at 20 g/kg *Spirulina* with the value of 86.11%. Bleakley and Hayes (2021) revealed that *Spirulina* inhibited ACE by 91.04% at concentration 1 mg/mL. *Spirulina* contains bioactive peptides derived from phycobiliproteins, which are known to have ACE-inhibitory activity (Anekthanakul et al. 2019). Angiotensin-converting enzyme (ACE) is essential hormone system responsible for controlling blood pressure (Lu et al. 2021). ACE inhibitory activity lowers the blood pressure by reducing the production of angiotensin II (Ma et al. 2019). The peptides inhibit ACE by binding with the active site of ACE via hydrogen bonds (competitive inhibition) or binding to the enzyme–substrate complex and the inhibitor does not compete with the substrate (non-competitive inhibition) (Lu et al. 2021). Similarly, tempeh has been reported to contain bioactive peptides which are responsible for the increase of ACE-inhibition activity. These peptides include alanin-valin, glycine-leucine, isoleucine-alanine-lysine, etc., which has ACE inhibitor activity (Tamam et al. 2019). This could be due to a combination of several compounds both in tempeh and *Spirulina* which improved the ACE-inhibitory activity.

Several studies has reported that microalgae exhibit antihypertensive properties due to its bioactive peptide content (Barkia et al. 2019; Ochoa-Méndez et al. 2016; Suetsuna and Chen 2001). Miczke et al. (2016) demonstrated a hypotensive effect by lowering the systolic and diastolic blood pressure after three months of *Spirulina* supplementation at a daily dose of 2 g to a total of 40 patients with hypertension. Another study by Martínez-Sámano et al. (2018) exhibited a significant decreased in systolic blood pressure from 140.0 to 126.5 mmHg after 12-weeks treatment of *Spirulina maxima* at a dose of 4.5 g per day. These antihypertensive effects depends on the microalgal species and peptides (Jiang et al. 2021). The consumption of *Spirulina* with valuable antioxidant and antihypertensive activity potentially lower the risk of cardiovascular disease and other metabolic disorders.

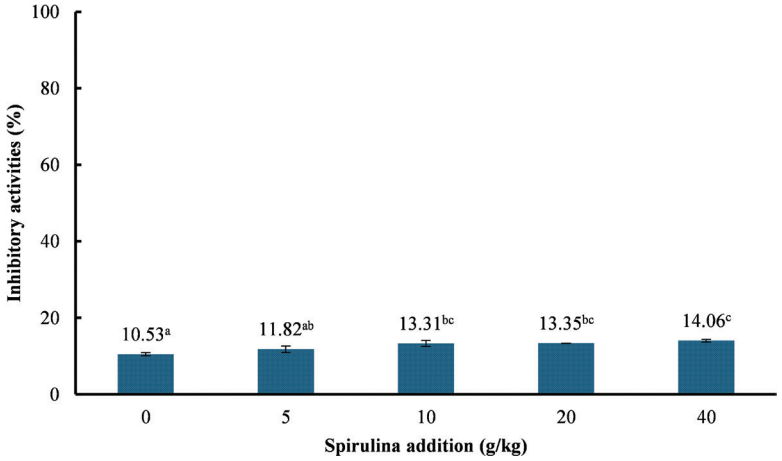

Figure 2. α-amylase inhibition activities of tempeh with the addition of *Spirulina* in five different concentrations.

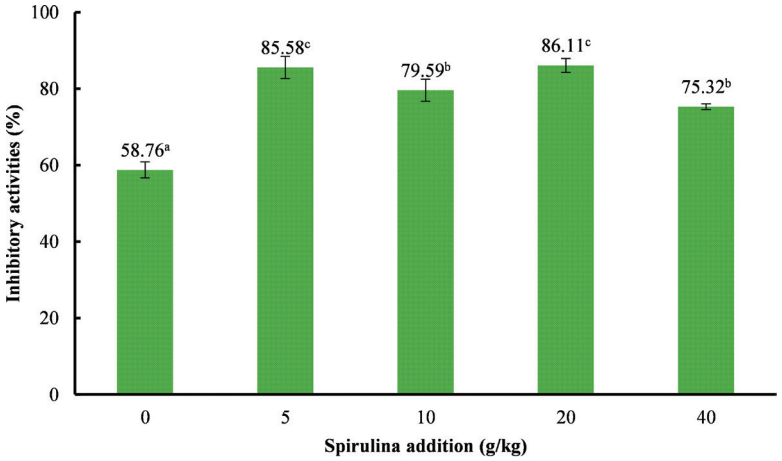

Figure 3. ACE inhibition activities of tempeh with the addition of *Spirulina* in five different concentrations.

According to antioxidant activity, it was found that the higher the *Spirulina* concentration and the longer the fermentation time, the higher the antioxidant activity due to the presence of antioxidative compounds, such as phenolic, phycocyanin and carotenoid. In regard to α-amylase inhibitor activity, it was found that the higher the *Spirulina* concentration and the longer the fermentation time, the higher the α-amylase inhibitor activity, which may be related to the prevention of oxidative stress by antioxidative compounds. Based on ACE inhibitor test, the results showed that the ACE inhibitor activity of all *Spirulina* tempeh was higher than the control due to the presence of bioactive peptides. These results indicate that the addition of *Spirulina* and the duration of fermentation have a significant effect on the functional properties of *Spirulina* tempeh, especially in the presence of bioactive peptides and phenolic compounds present. From this study, *Spirulina* tempeh has good potential to be developed as a functional food.

Nowadays, microalgae is commerciallized as a superfood supplement in numerous forms, such as powder, flakes or capsule. It can be either consumed directly or incorporated into various types of food (Lafarga et al. 2020). A number of earlier studies have investigated the effect of microalgae enrichment on many food products, such as ice cream, yogurt, cheese, kefir, cookies, bread, pasta and fermented soy drink (Barkallah et al. 2017; Csatlos et al. 2023; Falcão et al. 2023; Kahraman Ilıkkan and Bağdat 2023; Khemiri et al. 2020; Lucas et al. 2018; Niccolai et al. 2019; Şahin 2020; Szmejda et al. 2018). In general, the addition of microalgae (*Spirulina platensis*, *Chlorella vulgaris*, *Dunaliella salina*) enhance the physicochemical and the bioactivity properties, especially the antioxidant activity. In terms of the sensory acceptability, those products exhibited varied appreciations. For instance, the incorporation of 0.25% *Spirulina* improved the protein and dietary fiber content, the antioxidant activity, and the textural properties without negatively affecting the sensorial acceptance. On the contrary, the incorporation of *Spirulina* at concentration >7.5% increased the protein content of extruded snacks, but decreased the overall acceptability (Morsy et al. 2014).

There has been recent studies explored the synergistic potential of *Spirulina* with certain additives to further improve its functional properties. Jalili et al. (2024) reported the mutual effect of *Spirulina platensis* (3%), *Chlorella vulgaris* (1.467%), and curcumin (1%) in cheese, resulting in enhanced antioxidant activity and iron content with minimized amount of fat and acceptable sensory profile. *Spirulina platensis* and *Chlorella vulgaris* primarily affected the iron content and antioxidant activity, meanwhile curcumin had impacts on iron and phenolic content. Another study by Paternina et al. (2024) investigate the nutritional and functional synergism of *Spirulina* and acai in gummy candies, which exhibited synergistic effects in improving the nutritional content and antioxidant activity with positive sensory acceptance. Moreover, *Spirulina* also demonstrated synergistics interaction with *Bacillus subtilis* for enhancing folate production (Rehman et al. 2024). These provide new insights to stimulate the exploration and broaden the developmental prospect of *Spirulina* as functional food.

Sensory characteristics of *Spirulina* tempeh

In order to evaluate the sensory aspect, *Spirulina* tempeh was subjected to two different evaluations including intensity and hedonic test. The intensity of several attributes of *Spirulina* tempeh was evaluated by the panels and the results are presented in Table 4. Tempeh without addition of *Spirulina* was used as control. The results showed that the higher addition of *Spirulina* increased the intensity of the colour. This could be due to the phycocyanin pigment from *Spirulina* which is responsible for green blue colour. *Spirulina* is among the main sources of phycocyanin since it contained 54.65 mg phycocyanin/g biomass (Khandual et al. 2021). However, since it cannot be homogenously distributed during solid state fermentation in tempeh making, this causes a lower score of uniformity of the product colour along the increase of the concentration added to tempeh.

The addition of *Spirulina* affects the intensity of the taste. The higher the *Spirulina* added, the higher the bitter taste was detected. This might be due to bioactive pro-

Table 4. Profile of sensory attribute intensity of *Spirulina* tempeh.

Intensity	*Spirulina* addition (g/kg)				
	0	5	10	20	40
Colour	2.14 ± 1.77^{a}	3.42 ± 1.52^{b}	4.06 ± 1.82^{c}	4.84 ± 1.95^{d}	6.71 ± 2.04^{e}
Colour homogeneity	8.14 ± 1.11^{d}	6.30 ± 1.72^{bc}	5.71 ± 1.93^{ab}	5.27 ± 2.08^{a}	6.49 ± 2.20^{c}
Saltiness	6.01 ± 1.92^{ab}	6.22 ± 1.53^{b}	5.54 ± 1.83^{a}	5.48 ± 1.94^{a}	5.80 ± 1.99^{ab}
Umami	6.23 ± 1.64^{ab}	6.43 ± 1.44^{b}	6.11 ± 1.65^{ab}	5.77 ± 1.71^{a}	5.87 ± 1.89^{ab}
Bitterness	2.47 ± 1.88^{a}	2.79 ± 1.52^{a}	3.56 ± 2.26^{b}	4.16 ± 2.20^{bc}	4.51 ± 2.33^{c}
Fishy smell	2.44 ± 1.92^{a}	3.81 ± 1.91^{b}	4.30 ± 2.14^{b}	5.04 ± 2.23^{c}	6.23 ± 2.28^{d}
Rancidity	3.32 ± 2.42^{a}	3.48 ± 2.02^{a}	3.94 ± 2.13^{ab}	4.68 ± 2.11^{bc}	4.93 ± 2.30^{c}
Beany flavour	2.48 ± 2.40^{a}	3.89 ± 1.88^{b}	4.59 ± 2.19^{b}	5.65 ± 2.02^{c}	6.06 ± 2.25^{c}
Oily	4.74 ± 2.09^{ab}	5.43 ± 2.00^{b}	4.67 ± 1.80^{a}	4.83 ± 2.09^{ab}	4.64 ± 2.07^{a}
Firmness	6.35 ± 1.77^{a}	5.88 ± 1.71^{a}	6.30 ± 1.71^{a}	6.25 ± 1.82^{a}	6.12 ± 1.78^{a}
Compactness	7.21 ± 1.52^{b}	6.78 ± 1.48^{ab}	7.04 ± 1.48^{ab}	7.29 ± 1.28^{b}	6.62 ± 1.86^{a}
Raw tempeh colour	3.03 ± 1.94^{a}	5.19 ± 1.73^{b}	6.13 ± 1.53^{c}	6.78 ± 1.45^{d}	8.26 ± 1.11^{e}
Homogeneity of raw tempeh colour	8.21 ± 1.03^{b}	6.30 ± 1.83^{a}	6.03 ± 1.74^{a}	5.97 ± 2.07^{a}	6.06 ± 2.19^{a}

$^{a\text{-}e}$Values with different superscript alphabet in one column are significantly different based on Duncan's test ($p < 0.05$).

Table 5. Hedonic test of tempeh at different *Spirulina* concentrations.

Hedonic	*Spirulina* addition (g/kg)				
	0	5	10	20	40
Colour	8.01 ± 1.53^{d}	6.82 ± 1.51^{c}	6.30 ± 1.68^{c}	5.46 ± 2.12^{b}	4.23 ± 2.36^{a}
Colour homogeneity	8.15 ± 1.23^{d}	6.79 ± 1.51^{c}	6.19 ± 1.90^{bc}	5.87 ± 2.04^{ab}	5.46 ± 2.44^{a}
Saltiness	6.79 ± 1.85^{bc}	6.97 ± 1.58^{c}	6.23 ± 1.98^{ab}	6.01 ± 2.10^{a}	5.80 ± 2.28^{a}
Umami	6.86 ± 1.73^{c}	7.03 ± 1.49^{c}	6.57 ± 1.75^{bc}	5.96 ± 1.97^{ab}	5.68 ± 2.25^{a}
Bitterness	6.48 ± 2.57^{bc}	6.75 ± 2.15^{c}	5.83 ± 2.36^{ab}	5.32 ± 2.44^{a}	5.16 ± 2.59^{a}
Fishy smell	6.86 ± 2.40^{b}	6.51 ± 1.82^{b}	6.14 ± 2.27^{b}	5.26 ± 2.34^{a}	4.49 ± 2.60^{a}
Rancidity	6.12 ± 2.70^{c}	6.15 ± 2.24^{c}	5.87 ± 2.38^{bc}	5.01 ± 2.30^{a}	5.10 ± 2.43^{ab}
Beany flavour	6.17 ± 2.81^{c}	6.21 ± 1.99^{c}	5.56 ± 2.29^{bc}	5.00 ± 2.25^{ab}	4.49 ± 2.37^{a}
Oily	5.92 ± 2.32^{a}	5.73 ± 2.09^{a}	6.11 ± 1.96^{a}	5.52 ± 1.98^{a}	5.72 ± 2.08^{a}
Firmness	6.83 ± 1.63^{a}	6.81 ± 1.70^{a}	6.64 ± 1.85^{a}	6.22 ± 1.98^{a}	6.39 ± 1.78^{a}
Compactness	7.20 ± 1.36^{b}	6.94 ± 1.75^{b}	6.87 ± 1.71^{b}	6.22 ± 1.92^{a}	6.75 ± 1.71^{ab}
Colour appearance	8.08 ± 0.97^{d}	6.89 ± 1.18^{c}	6.50 ± 1.55^{c}	5.83 ± 1.87^{b}	5.13 ± 2.32^{a}
Raw tempeh colour	7.89 ± 1.34^{e}	6.51 ± 1.73^{d}	5.26 ± 1.86^{c}	4.38 ± 1.96^{b}	3.74 ± 2.37^{a}
Homogeneity of raw tempeh colour	8.21 ± 1.03^{c}	6.76 ± 1.64^{b}	5.76 ± 1.94^{a}	5.16 ± 1.94^{a}	5.55 ± 2.25^{a}
Overall likeness	7.54 ± 1.27^{d}	6.98 ± 1.27^{c}	6.21 ± 1.49^{b}	5.59 ± 1.62^{a}	5.07 ± 2.30^{a}

$^{a-c}$Values with different superscript alphabet in one column are significantly different based on Duncan's test ($p < 0.05$).

teins and peptides in *Spirulina* which have been reported to be responsible for bitter taste (Rajmohan and Bellmer 2019). On the other hand, the umami and saltiness are not affected. Although *Spirulina* is naturally salty, it is added at low concentrations, which may not be not be noticeable to the panelists, especially since 7% brine was used to marinate all samples prior frying. The addition of *Spirulina* increases the intensity of fishy smell, rancidity, and beany flavors. The higher *Spirulina* added, the higher the afore-mentioned unpleasant odour intensity. The fishy smells could be due related to the mineral content of the *Spirulina*. *Spirulina* contains ash as much as 23.08% (Mostolizadeh et al. 2020). The rancidity odour could be related to long-chain polyunsaturated fatty acids of *Spirulina*, which can be oxidized and release rancidity flavour (Abbas et al. 2021). These unpleasant odours become the limit of *Spirulina* addition. The addition of *Spirulina* did not significantly affect the texture of tempeh, such as firmness and compactness. One possible reason for this could be the low concentration of *Spirulina*, which was insufficient to alter the structure of *Spirulina* tempeh.

The hedonic test showed that in general, the hedonic score decreased along with the increase concentration of *Spirulina* added to the tempeh (Table 5). The significant decrease of the hedonic score was observed in colour attributes which decline from like very much for the control to dislike slightly at 40 g/kg *Spirulina* added. With regard of colour, the maximum *Spirulina* added is 10 g/kg which resulted in 6.30 score (light slightly). This result is in accordance with the intensity attribute test showed that higher *Spirulina* added caused higher intensity of the colour which decrease the hedonic score since panels perceived the colour of tempeh should be white. Similarly, the hedonic score of fishy smells, rancidity, and beany flavors also declined with the elevating concentrations of *Spirulina*. The lower hedonic score is due to the increasing intensity of the unpleasant odours. The maximum amount of *Spirulina* added to tempeh was 10 g/kg, which produced tempeh that was liked slightly by the panelists. No difference was observed on the hedonic score of oily, firmness and compactness of tempeh. This is in accordance with the similar score of intensity of these attributes which shows that addition of *Spirulina* did not affect these attributes. The result showed that the overall likeness of *Spirulina* tempeh decreased with the increase of *Spirulina*. The maximum *Spirulina* added to the tempeh is 10 g/kg and this produces tempeh which like slightly by the panels.

Conclusions

Fermentation time affects the functionality of Spirulina tempeh. The best fermentation time to obtain the highest functionality is 48 h. Addition of Spirulina could enhance the functional properties of tempeh. The antioxidant, antidiabetic and antihypertension increase by 39–104.7, 12.2–33.5 and 28.2–46.5% with addition of 5–40 g/kg of *Spirulina*. However, it is not desirable in terms of sensory, which could reduce the hedonic score from like very much to dislike slightly, especially for the colour and odour. Therefore, the optimum concentration of *Spirulina* in terms of functionality and sensory is 20 g/kg. This study reveals that *Spirulina* tempeh is a potential functional food product due to its valuable protein and bioactive compounds. Further investigation is needed to understand the functional effect, such as antidiabetic, antihypertensive and anti-inflammatory effects of food products enriched with *Spirulina*.

Author contributions

Rachma Wikandari: Conceptualization, funding acquisition, supervision, writing – original draft and writing – review and editing. Jocelyn Tanya: Data curation and formal analysis. Nadia Salwa Rahmadina: Data curation and formal analysis. Atris Suyantohadi: Funding acquisition and writing – review and editing. Dwi Larasatie Nur Fibri: Data curation and supervision.

Conflict of interest

The authors declare that there is no conflict interest in the publication.

Acknowledgements

This work was financially supported by Faculty of Agricultural Technology, Gadjah Mada University through Synergy Research Grant 2020 [grant number 1850/UN1/FTP.1.3/SET-D/KU/2020].

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