



AMINO ACID PROFILE AND ANTIOXIDANT PROPERTIES OF TEMPEH FORTIFIED WITH SEAWEED *Euचेuma spinosum*

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Abstract

Indonesia is the second largest seaweed producer in the world. One seaweed species, *Euचेuma spinosum*, contains various bioactive compounds that act as antioxidants. Tempeh is a traditional Indonesian fermented food that is rich in nutrients and contains all essential amino acids. Therefore, fortifying tempeh with *E. spinosum* may enhance its nutritional value, antioxidant properties, and functional potential. This study aimed to determine the effect of adding *E. spinosum* on the amino acid profile and antioxidant properties of tempeh and identify the optimal level of *E. spinosum* addition. The research was conducted using a completely randomized design with four treatments: control (T0), 10% (T1), 20% (T2), and 30% (T3) *E. spinosum* added to soybean tempeh. Tempeh with 30% seaweed exhibited the highest soluble protein content (24.07 mg BSAE/g), strong antioxidant activity (109.4 ppm), total phenol content (11.01 mg GAE/g), and the most favorable chewiness texture. HPLC analysis of amino acids revealed that the control tempeh had the highest amino acid levels, whereas seaweed addition generally reduced amino acid content. FTIR analysis indicated changes in protein structure in seaweed-fortified tempeh, as shown by shifts in absorption bands in the FTIR spectrum corresponding to the main functional groups. Overall, *E. spinosum* fortification significantly affected the soluble protein content, antioxidant activity, total phenol levels, texture, and amino acid content of the fortified tempeh.

Keywords: bioactive compound, fermentation, functional food, protein, soybeans

Profil Asam Amino dan Sifat Antioksidan Tempe yang Difortifikasi dengan Rumput Laut *Euचेuma spinosum*

Abstrak

Indonesia merupakan penghasil rumput laut terbesar kedua di dunia. *Euचेuma spinosum* merupakan salah satu rumput laut yang mengandung berbagai senyawa bioaktif yang berperan sebagai antioksidan. Tempe merupakan makanan fermentasi tradisional Indonesia yang kaya nutrisi dan mengandung semua asam amino esensial. Oleh karena itu, fortifikasi tempe dengan *E. spinosum* dapat meningkatkan nilai gizi, sifat antioksidan, dan potensi pangan fungsionalnya. Penelitian ini bertujuan untuk mengetahui pengaruh penambahan *E. spinosum* terhadap profil asam amino dan sifat antioksidan tempe, serta untuk mengetahui penambahan *E. spinosum* yang terbaik. Penelitian ini menggunakan rancangan acak lengkap dengan empat perlakuan, yaitu kontrol (T0), 10% (T1), 20% (T2), dan 30% (T3) *E. spinosum* yang ditambahkan ke dalam tempe kedelai. Tempe dengan 30% rumput laut menunjukkan kandungan protein terlarut tertinggi (24,07 mg BSAE/g), aktivitas antioksidan IC₅₀ (109,4 ppm), dan kandungan fenol total (11,01 mg GAE/g), serta tekstur kenyal yang paling disukai. Analisis HPLC asam amino menunjukkan bahwa tempe kontrol memiliki kadar asam amino tertinggi, sementara penambahan rumput laut secara umum menurunkan kadar asam amino. Analisis FTIR menunjukkan perubahan struktur protein pada tempe yang diperkaya rumput laut, ditunjukkan oleh pergeseran pita serapan pada spektrum FTIR yang sesuai dengan gugus fungsi utama.

Secara keseluruhan, fortifikasi *E. spinosum* secara signifikan memengaruhi kandungan protein terlarut, aktivitas antioksidan, kadar fenol total, tekstur, dan kandungan asam amino tempe.

Kata kunci: fermentasi, kedelai, komponen bioaktif, pangan fungsional, protein

INTRODUCTION

Indonesia is the second-largest seaweed producer in the world. Seaweed production in Indonesia reached 9.6 million tons in 2022 (KKP, 2023). Currently, seaweed is used to improve food texture, as herbal medicine, fertilizer, fungicide, herbicide, biopolymer, and raw material for the pharmaceutical industry (Olsson *et al.*, 2020). Seaweed is a nutritious food source because it contains vitamins, proteins, minerals, fiber, unsaturated fatty acids, essential fatty acids, and macro- and micronutrients beneficial for humans, such as antioxidants and anti-inflammatory agents (Nurshahida *et al.*, 2020; Dewi *et al.*, 2025a). *Euचेuma spinosum* is widely cultivated and utilized in Indonesia. The red seaweed *E. spinosum* contains a high amount of polysaccharides but is low in calories.

However, *E. spinosum* has not been widely utilized because of its high viscosity and large molecular weight (Tuwo *et al.*, 2021; Zhang *et al.*, 2023). *E. spinosum* is a major source of carrageenan, which is used as a thickener, stabilizer, and gel-forming agent in the food industry (Diharmi *et al.*, 2017). In addition, *E. spinosum* has a high nutritional content and bioactive compounds, making it promising for enhancing the functional value of food products when used as a fortification ingredient (Damongilala *et al.*, 2021). Bioactive compounds in *E. spinosum*, such as polyphenols, flavonoids, pigments (phycobiliproteins), and carotenoids, are known to exhibit antioxidant activity (Ariano *et al.*, 2021). Hradaya & Husni (2021) reported that *E. spinosum* contains 170.02 mg GAE/g total phenolics, 789.21 mg GAE/g total phenol, and an antioxidant activity IC_{50} of 362.52 ppm. Antioxidants play a crucial role in neutralizing free radicals, preventing oxidative damage, and reducing the risk of degenerative diseases (Chakraborty & Santra, 2017). The antioxidant content of *E. spinosum* has potential applications in fortifying commercial food products.

Tempeh is a fermented soybean product made using *Rhizopus* sp. fungi and is widely enjoyed by the Indonesian population owing to its pleasant taste and high nutritional content (Aryanta, 2020). The nutritional content of 100 g of tempeh includes 20.8 g of protein, 8.8 g of fat, 1.4 g of fiber, 155 mg of calcium, 326 mg of phosphorus, 4 mg of iron, 0.91 mg of vitamin B1, and 34 μ g of carotenoids (Syarfaini *et al.*, 2019). Tempeh is rich in plant protein, is easily digestible, and has a well-balanced amino acid profile, including essential amino acids required by the body (Fertiasari *et al.*, 2024). Research on tempeh has widely developed using raw materials other than soybean. Tan *et al.* (2024) who produced soy tempeh substituted with chickpeas and red beans. Kim *et al.* (2025) modified tempeh using chickpeas, which are rich in carbohydrates and protein. Thulesen *et al.* (2025) made tempeh from faba beans to increase the nutritional content of tempeh, while Castaneda *et al.* (2025) made tempeh from faba beans and whole grain oats to increase the protein and dietary fiber content of tempeh.

These studies show that the success rate of the resulting tempeh characteristics is influenced by the raw materials and tempeh production process used, especially in the preparation of raw materials and fermentation process (Chauhan *et al.*, 2022; Reale *et al.*, 2025). Tempeh fermentation involves enzymatic activity that can modify the matrix of the raw material and alter biochemical components, such as increasing the nutritional value and functional properties of the product (Harahap *et al.*, 2026). During tempeh fermentation, *Rhizopus* forms mycelium that grows inside and envelops the outside of tempeh, allowing the mycelium to bind raw materials, such as soybeans, and form a solid matrix. This affects the texture of the resulting tempeh.

Furthermore, *Rhizopus* produces enzymes that degrade carbohydrates and proteins into simpler components. These



compounds alter the physicochemical properties and nutritional content of the resulting tempeh. Furthermore, nutrients such as amino acids and peptides produced during fermentation play an important role in increasing the antioxidant content of tempeh (Hernandez *et al.*, 2017; Wang *et al.*, 2023; Kim *et al.*, 2025). Several studies have reported that the fermentation of foods high in polysaccharides can increase their antioxidant content (Qi *et al.*, 2024; Wang *et al.*, 2024; Li *et al.*, 2025).

Fortifying tempeh with *E. spinosum* represents an innovative approach to enhance the nutritional value and functional quality of the product. The combination of tempeh, which is rich in protein, with *E. spinosum*, which is rich in polysaccharides and bioactive compounds, results in a food that is not only nutritious but also health-promoting, with an improved amino acid profile and stronger antioxidant activity. Amrizal *et al.* (2020) found that nori with higher concentrations of *E. spinosum* had higher antioxidant content and better product texture. Muhtar *et al.* (2019) reported that adding 7% *E. spinosum* produced a jelly drink containing antioxidants such as flavonoids, terpenoids, alkaloids, ascorbic acid, phenols, and phlorotannins. However, to date, no studies have applied *E. spinosum* to tempeh or evaluated the changes in its amino acid profile and antioxidant properties. This study aimed to determine the effect of adding *E. spinosum* on the amino acid profile and antioxidant properties of tempeh and to identify the best level of *E. spinosum* addition.

MATERIALS AND METHODS

Production of Seaweed-Fortified Tempeh

The method for producing seaweed-fortified tempeh was based on that of Dewi *et al.* (2025b), with modifications in the source of *E. spinosum*. *E. spinosum* was obtained from seaweed farmers in Jepara, Central Java, Indonesia (Figure 1). White soybeans and Raprima tempeh starter were obtained from a traditional market in Semarang, Central Java, Indonesia. Raprima consist of rice flour 99% and *Rhizopus oligosporus* 1%. First, *E. spinosum* samples were prepared for analysis. The seaweed was washed thrice to remove impurities. The samples were then soaked in water at a ratio of 1:15 for 3 h. After soaking, the seaweed was blanched in boiling water for 4 min and then blended until smooth. Second, tempeh production was performed. Soybeans were washed and boiled at a ratio of 2:1 (water: soybeans) for 30 min.

The soybeans were then soaked in the boiled water residue for 24 h. The outer skin was removed by squeezing the soybeans until the skins separated, leaving the soybean kernels without the skin. The soybeans were washed and steamed for 30 min. Next, 0.1% tempeh starter was added based on the soybean weight and mixed thoroughly. *E. spinosum* was added at concentrations of 0% (T0), 10% (T1), 20% (T2), and 30% (T3), with three replicates. The seaweed was mixed thoroughly until fully incorporated, then wrapped in plastic, sealed, and punctured with a toothpick at intervals of approximately 1 cm. Fermentation was carried out by placing raw tempeh on a



Figure 1 *Eucheuma spinosum*

perforated rack at room temperature for 48 h. The fermented tempeh was tested.

Soluble Protein

This test was conducted using the Bradford method, with Coomassie Brilliant Blue (CBB) as the indicator. CBB binds to proteins, causing a color change to blue, which was measured using a visible spectrophotometer (PG Instruments Ltd., UK) at wavelengths of 465–595 nm, using bovine serum albumin (BSA) as the standard (Mardhika *et al.*, 2020).

Antioxidant Activity

Antioxidant activity was assessed using the DPPH method and expressed as % inhibition and IC_{50} /ppm using α, α -diphenyl- β -picrylhydrazyl (DPPH). Samples were dissolved in methanol at different concentrations, and 0.1 mM DPPH reagent was added to the solution. The mixture was incubated at room temperature in the dark for 30 min. After incubation, the absorbance was measured using a spectrophotometer (T70 UV-Vis, PG Instruments Ltd., UK) at 517 nm. The percentage inhibition (PI) of DPPH radicals was calculated as follows:

$$\text{Radical DPPH (PI)} = [(Ab - As)/Ab] \times 100$$

Where Ab refers to the absorbance of the control (without the sample) and As refers to the absorbance of the sample.

Antioxidant activity was reported in two parameters: % inhibition at a specific concentration and IC_{50} (ppm), defined as the sample concentration required to inhibit 50% of the DPPH radicals (Salem *et al.*, 2017; Beyazen *et al.*, 2017).

Total Phenol

Total phenol was measured using the Folin-Ciocalteu method to evaluate the phenolic content in the samples. One milliliter of FC reagent was mixed with the sample for 5 min, and then 10 ml of 7.5% sodium carbonate solution was added and mixed. The final volume was adjusted to 25 ml with deionized water and allowed to stand for 1 h. Phenolic content was analyzed at 750 nm using a spectrophotometer (Shimadzu, Japan)

based on a calibration curve prepared with standard compounds (gallic acid, 0–200 mg/ml). The final quantitative result was expressed as mg gallic acid equivalents (GAE) per 100 g of fresh weight (Alkaltham *et al.*, 2021).

Texture

Texture testing was performed to measure the hardness, cohesiveness, springiness, gumminess, chewiness, adhesiveness, and fracture force of tempeh. The measurements were performed using a texture analyzer by pressing the sample at a specific speed. The tempeh was placed in a prepared container and pressed with a round probe of 0.5 inch diameter (Rochmah *et al.*, 2019).

Amino Acid Profile

This test was conducted using ultra-performance liquid chromatography (UPLC) (Waters Corporation, USA). The amino acid score was used to assess how well the amino acids in a food product can be absorbed and utilized by the body. The score was calculated by comparing the amino acid content of the sample with that of the standard essential amino acid pattern. The standard essential amino acid requirements were as follows: histidine (1.9), lysine (5.8), threonine (3.4), isoleucine (2.8), leucine (6.6), valine (3.5), methionine + cysteine (2.5), phenylalanine + tyrosine (6.3), and tryptophan (1.1).

$$\text{SAA} = \text{AAE sample} / \text{AAE standard} \times 100\%$$

Where SAA is the Amino Acid Score and AAE is the Essential Amino Acid content (Riviani *et al.*, 2020).

FTIR

Fourier-transform infrared spectroscopy (FTIR) (Perkin Elmer, USA) was performed on dried and powdered tempeh. Eight scans were accumulated in the transmission mode with a resolution of 4 cm^{-1} . The spectrum was recorded from 4,000 to 600 cm^{-1} (Gullón *et al.*, 2017).

Statistical Analysis

This study used a completely randomized design with a single factor,



namely, the addition of *E. spinosum* at different concentrations. The study was performed in triplicates. Data were analyzed using analysis of variance (ANOVA). If a significant difference was found ($p < 0.05$), Duncan's post hoc test was performed. Data were processed using SPSS IBM 23.

RESULT AND DISCUSSION

Soluble Protein

Soluble proteins refer to simple protein components (oligopeptide group) consisting of amino acid chains shorter than ten, making them more water-soluble than larger proteins due to their shorter chain length (Prihatiningsih *et al.*, 2021). Table 1 shows that sample T3 had the highest soluble protein content at 24.07%, while the addition of 10% seaweed (T1) showed no significant difference from that of the control. T3 treatment significantly increased the soluble protein content, and higher concentrations of seaweed led to higher soluble protein levels. This result aligns with that of Kusnandar *et al.* (2020), who found that tempeh made from red beans had soluble protein levels of approximately 20–27%.

Red seaweed has a higher protein content than green and brown seaweeds. Red seaweed contains approximately 150–200 g/kg dry weight, green seaweed 90–180 g/kg dry weight, and brown seaweed 50–120 g/kg dry weight (Olsson *et al.*, 2020). Fortification with *E. spinosum* increases the soluble protein content in tempeh due to the direct addition of protein/peptide fractions from seaweed (Brien *et al.*, 2022). The increase in soluble protein content in tempeh is also due to fermentation. Microbial proteolytic activity during fermentation enhances the release of soluble

peptides (Damongilala *et al.*, 2021). During fermentation, *Rhizopus* produces proteolytic enzymes that hydrolyze proteins by breaking peptide bonds, resulting in amino acids and short-chain polypeptides. The breakdown of peptide bonds by this proteolytic activity is associated with an increase in soluble protein content (Puspitojati *et al.*, 2019; Ishartani *et al.*, 2021). The results of this study are consistent with those of Ishartani *et al.*, who produced tempeh from lamtoro seeds and found that the soluble protein content increased as the amount of seeds increased.

Antioxidant Activity

The antioxidant activity IC_{50} value decreased with increasing *E. spinosum* concentrations. The highest IC_{50} value was observed in T0 (146.9 ppm), whereas the lowest was observed in T3 (109.4 ppm). This indicates that the ability of the product to capture or neutralize free radicals increased. Lower IC_{50} values indicate a stronger antioxidant potential (Gulcin and Alwasel, 2023). This is supported by the increasing % inhibition, where higher *E. spinosum* concentrations led to higher radical scavenging activity. The results of this study were higher than those reported by Evangelista and Surya (2024), who found that tempeh with 0.7% butterfly pea flowers had an antioxidant activity of 82.94%. However, the results were lower than those reported by Yudiono (2023), who found that tempeh with Moringa leaf flour had an antioxidant activity of 93.67%. As shown in Table 1, the highest radical scavenging activity was observed in T3 (86.64%) and the lowest in T0 (63.25%). These values are consistent with the IC_{50} results, where lower IC_{50} values indicate that a smaller sample size produces strong antioxidant

Table 1 Analysis of soluble protein, DPPH, and total phenol in tempeh fortified with *E. spinosum*

Sample	Soluble Protein %	DPPH (ppm)	DPPH (% inhibition)	Total Phenol (mg GAE/g)
T0	22.55±0.01 ^a	146.9±0.12 ^d	63.25	9.34±0.01 ^a
T1	22.60±0.02 ^a	129.3±0.43 ^c	71.54	9.53±0.00 ^a
T2	22.93±0.02 ^b	128.7±0.07 ^b	75.88	10.26±0.37 ^b
T3	24.07±0.08 ^c	109.4±0.07 ^a	86.64	11.01±0.38 ^c

Data within the same column that have different superscripts indicate significant differences ($p \leq 0.05$)

effects (Ziemlewska *et al.*, 2021). Fortification of tempeh with *E. spinosum* improved its antioxidant activity. This enhancement is attributed to the presence of bioactive compounds in *E. spinosum*, such as phenolic compounds, flavonoids, and carotenoid pigments (Sofiana *et al.*, 2020). Additionally, soybeans produce isoflavones through fermentation. Isoflavones are known to act as antioxidants and can function synergistically with other antioxidants (Kuligowski *et al.*, 2017). Fermentation of grains, vegetables, and other plants using starter fungi, lactic acid bacteria, or yeast increases the levels of phenolic compounds, flavonoids, vitamins, and bioactive peptides. Microbial growth produces enzymes such as β -glucosidase, esterase, and protease, which break down cell walls and hydrolyze related compounds, releasing bioactive compounds into the medium and enhancing the radical scavenging capacity (Hur *et al.*, 2014). The combination of two different antioxidant sources has the potential to enhance the overall effectiveness of the product in neutralizing free radicals, making *E. spinosum*-fortified tempeh more effective than regular soybean tempeh in this regard. The antioxidant activity results of this study are consistent with the increased total phenol content. A high total phenol content has a linear effect on high antioxidant activity. Similar results were obtained by Yudiono *et al.* (2021), who produced tempeh using various types of soybeans.

Total Phenol

The total phenol content increased with higher concentrations of *E. spinosum*. This indicates that fortification with *E. spinosum* enriched the bioactive components of tempeh. Tempeh without *E. spinosum* (T0) had the lowest phenolic content at 9.34 mg GAE/g, while tempeh with 30% *E. spinosum* (T3) had a phenolic content of 11.01 mg GAE/g. Phenolic compounds act as antioxidants by donating hydrogen atoms to neutralize free radicals, thereby stopping chain reactions that could damage lipids or proteins in the body (Shahidi & Ambigaipalan, 2015). The increase in total phenol content in fermented products (tempeh) is directly related to

higher antioxidant activity (Xiao *et al.*, 2021). This study's total phenol content was higher than that reported by Šulc and Rysová (2025), who found tempeh made from a 2:1 mixture of yellow peas and sorghum had total phenol of 10.14 mg GAE/g, and tempeh made from a 1:2 mixture of quinoa and sorghum had 4.68 mg GAE/g. This indicates that *E. spinosum* effectively increased the phenolic content of tempeh and enhanced its antioxidant properties. The increase in total phenol content was due to the high levels of phenolic compounds and various secondary metabolites, such as polyphenols, flavonoids, and tannins, which act as antioxidants (Syakri *et al.*, 2024). During tempeh fermentation, total phenol content tends to increase due to the presence of β -glucosidase and esterase, which hydrolyze complex compounds into free phenolic forms (Zhao *et al.*, 2021). The presence of *E. spinosum* may also exert a synergistic effect with soybean isoflavones, enriching the total phenol content. Therefore, the higher the concentration of *E. spinosum* used, the higher the total phenol content in the tempeh product.

Texture

Table 2 shows that the addition of *E. spinosum* significantly affected the tempeh texture. The hardness decreased with increasing seaweed concentration. The highest hardness was observed in T0 (0.65 kgf), and the lowest in T3 (0.13 kgf). This indicates that the tempeh texture became softer with increasing *E. spinosum* levels. The hardness values in this study were lower than those reported by Sundari *et al.* (2024), where soybean tempeh combined with groundnut had hardness values of 12–14 kgf. Fresh tempeh is firm and soft. Increasing *E. spinosum* concentration improved cohesiveness and springiness, although these values were lower than those of the control. Cohesiveness measures product compactness, whereas springiness indicates the degree of elasticity or resilience, where a sample returns to its original shape (Danella, 2024). In this study, the cohesiveness and springiness values decreased. These values are consistent with those reported by Wikandari *et al.* (2020), who reported that tempeh made

Table 2 Texture of tempeh fortified with *E. spinosum*

Sample	Hardness (kgf)	Cohesiveness	Springiness (mm)	Gumminess (kgf)	Chewiness (kgf·mm)	Fracture force (kgf)	Adhesiveness (kgf·mm)
T0	0.65±0.07 ^d	0.96±0.04 ^c	6.11±1.00 ^c	0.11±0.02 ^b	0.95±0.00 ^b	0.09±0.00 ^a	0.28±0.01 ^c
T1	0.37±0.00 ^c	0.01±0.00 ^a	2.80±0.31 ^a	0.16±0.00 ^c	0.02±0.00 ^a	0.77±0.02 ^b	0.02±0.00 ^a
T2	0.20±0.03 ^b	0.12±0.03 ^b	9.77±0.00 ^d	0.11±0.01 ^b	0.96±0.00 ^b	0.09±0.00 ^a	0.06±0.07 ^b
T3	0.13±0.04 ^a	0.13±0.04 ^b	4.38±0.30 ^b	0.06±0.00 ^a	1.26±0.00 ^c	0.09±0.00 ^a	0.03±0.00 ^a

Data with different superscripts denote significant differences ($p \leq 0.05$), while data sharing the same superscript indicate no significant difference.

from various legumes had cohesiveness values of 0.45–0.61 and springiness values of 0.85–0.91 mm. The gumminess, chewiness, and adhesiveness decreased in sample T3, with values of 0.06 kgf, 1.26 kgf·mm, and 0.03 kgf·mm, respectively, indicating that the sample was easier to chew and less sticky, whereas the fracture force remained relatively stable.

Overall, fortification with *E. spinosum* (Figure 2) reduced the hardness and springiness but increased the elasticity and improved the sensory properties. Texture testing affects the physical characteristics of fresh and processed tempeh. If the texture values are too low, the tempeh may easily break, become brittle, or lack firmness, making it difficult to cut. If the texture values are too high, the tempeh will have poor texture quality due to excessive hardness, making it difficult to consume (Sundari *et al.*, 2024). The higher the concentration of *E. spinosum*, the softer, more elastic, and easier-to-chew the tempeh. This is due to the presence of natural hydrocolloids in *E. spinosum*, particularly carrageenan, which can bind water and form a gel structure, thereby increasing the moisture and elasticity of the product (Dewi *et al.*, 2025b). In addition, the soluble fiber

and polysaccharide components in seaweed can influence the functional characteristics of food, such as water-holding capacity, thereby reducing the density of the soybean matrix and resulting in a smoother and less brittle tempeh structure (Peñalver *et al.*, 2020). Seaweed also helps retain moisture during fermentation, reduces mass loss, and creates a soft texture (Syakri *et al.*, 2024).

Amino Acid Profile

The analysis of the amino acid profile is important because it can indicate whether a material meets the essential amino acid requirements for humans according to WHO standards and help in understanding changes in protein composition resulting from processing methods such as fermentation (Melini & Melini, 2021). In addition, several amino acids, such as glutamate and aspartate, play a role in developing the umami (savory) taste in fermented food products such as tempeh (Purwandari *et al.*, 2025). The amino acid profiles of the tempeh samples are presented in Table 3.

The most dominant amino acids in soybeans and *E. spinosum* were L-glutamic acid at 5,7941.98 mg/kg and 1,289.46 mg/kg, respectively, followed by L-aspartic acid at



Figure 2 Tempeh fortified with *E. spinosum* (%); (A) 0, (B) 10, (C) 20 and (D) 30

Table 3 Amino acid profile of tempeh fortified with *E. spinosum*

Amino acid type	Soybean (mg/kg)	<i>E. spinosum</i> (mg/kg)	Sample with fortification of <i>E. spinosum</i> (mg/kg)			
			0 (T0)	10 (T1)	20 (T3)	30 (T4)
L-Serine	22,206.14	885.88	6,408.77	4,966.01	4,277.96	3,780.5
L-Glutamic Acid	57,941.98	1,289.46	18,850.1	14,999.18	13,575.68	10,258.2
L-Phenylalanine	25,072.46	797.22	8,412.99	6,384.66	6,358.01	5,560.4
L-Isoleucine	15,219.57	572.48	5,695.35	4,368.66	3,739.45	3,346.915
L-Valine	15,748.21	786.73	6,228.25	4,956.61	4,187.2	3,785.67
L-Alanine	13,965.33	787.43	9,318.32	6,806.50	6,267.36	4,715.38
L-Arginine	29,738.3	772.09	6,753.22	5,691.41	4,810.22	4,299.45
Glycine	17,690.21	773.30	6,735.06	5,232.22	4,506.31	3,986.01
L-Lysine	18,120.63	298.69	4,869.14	4,155.75	3,336.40	2857
L-Aspartic Acid	32,649.19	1,226.22	9,616.09	7,648.08	7,259.82	5,466.67
L-Leucine	28,625.63	1,089.53	10,584.95	8,167.45	6,979.09	6,163.82
L-Tyrosine	14,499.15	n.d.	5,416.40	4,097.09	4,165.63	3,780.71
L-Proline	18,483.35	718.155	5,869.85	4,617.6	4,014.74	3,052.95
L-Threonine	16,731.43	886.36	5,456.05	4,059.17	3,544.32	3,111.29
L-Histidine	11,732.64	n.d.	4,307.74	3,138.54	3,108.40	2,560.55

32,649.19 mg/kg in soybeans and 1,226.22 mg/kg in *E. spinosum*. L-Glutamic and L-aspartic acids contribute to the umami flavor of food products (Amaliah *et al.*, 2024). Based on Table 3, the amino acid levels in tempeh with added *E. spinosum* (T1, T2, and T3) were generally lower than those in tempeh without *E. spinosum* (T0). T0 had L-glutamic acid at 18850.1 mg/kg, which decreased progressively with the addition of *E. spinosum*, reaching 10,258.2 mg/kg in T3. T0 in this study had higher L-glutamic acid than Wikandari *et al.* (2020), who reported 14,241.77 mg/kg in green bean tempeh, but lower than tempeh made from peanuts (53,370.99 mg/kg).

The decrease in amino acids may be due to the degradation of soybean protein during fermentation and interactions with sulfate polysaccharides from seaweed, which may inhibit the release of free amino acids (Ghelichi & Jacobsen, 2025). Another possibility is that during fermentation, the addition of *E. spinosum* catalyzes increased microbial growth, thereby boosting the production of proteolytic enzymes. This leads to a greater

breakdown of peptide bonds, resulting in an increased quantity of short-chain peptides. This is related to the increasing levels of soluble proteins (Surya *et al.*, 2024). Soluble proteins are important components of food products because they play a role in texture formation, affect the functional properties of proteins, and influence the digestibility of food (Anyiam *et al.*, 2025; Grossmann & McClements, 2023). Although it decreases the amino acid content of tempeh, the addition of *E. spinosum* results in higher polyphenol and flavonoid content, which enhances antioxidant activity. Other studies have also shown that adding *E. spinosum* increases the dietary fiber content of tempeh (Dewi *et al.*, 2025b).

FTIR Analysis

Fourier Transform Infrared Spectroscopy (FTIR) is a vibrational spectroscopy technique that measures the interaction between infrared radiation and the molecules in a sample, producing an absorption spectrum that reflects the chemical functional groups and molecular bonds

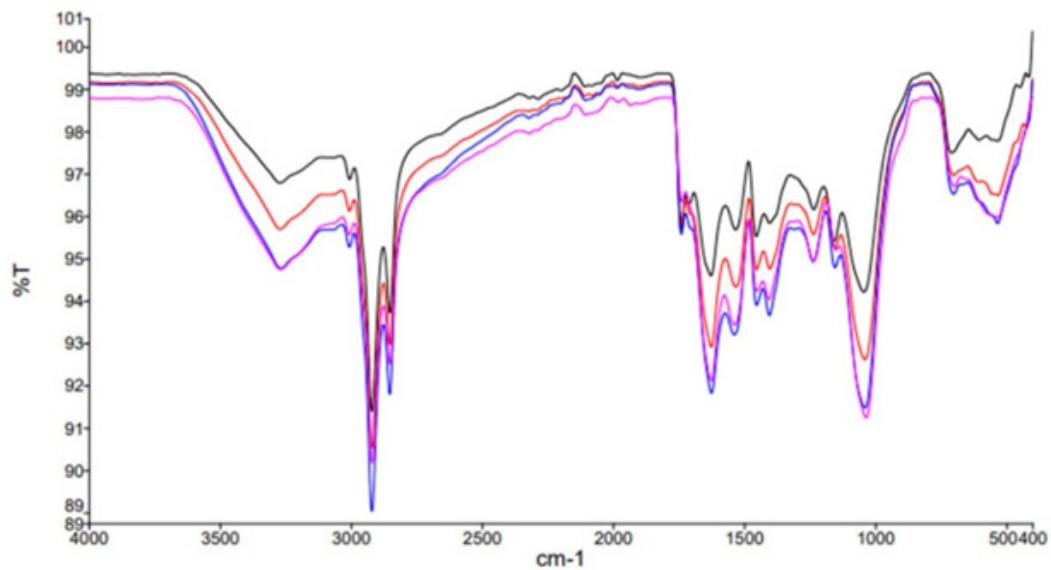


Figure 3 FTIR graphic analysis; T0 (—), T1 (—), T2 (—), T3 (—)

present in the substance (Li *et al.*, 2019). The FTIR spectra of tempeh with the addition of seaweed are presented in Figure 3.

The FTIR spectrum of soybeans showed characteristic peaks at $1,650\text{ cm}^{-1}$ (Amide I), $1,540\text{ cm}^{-1}$ (Amide II), and $1,240\text{ cm}^{-1}$ (Amide III) from the protein, while *E. spinosum* showed a strong peak at $\sim 1,220\text{--}1,260\text{ cm}^{-1}$ (S=O group from sulfated carrageenan). Soybeans were dominated by protein, whereas *E. spinosum* was dominated by sulfated polysaccharides. Tempeh with *E. spinosum* fortification showed that in the O-H and N-H stretching region ($3,400\text{--}3,200\text{ cm}^{-1}$), sample T0 had a strong peak around $3,400\text{ cm}^{-1}$, indicating hydrogen bonding in hydroxyl (O-H) and amino (N-H) groups. In samples T1, T2, and T3, the peak intensity decreased, indicating a reduction in free hydroxyl and amino groups, likely due to their interactions with seaweed components. According to Kedang *et al.* (2024), a strong absorption band around $\sim 3,300\text{ cm}^{-1}$ indicates the presence of -OH and -NH stretching vibrations derived from the hydroxyl and amide groups of polysaccharides and proteins, respectively (amide A band). In the C-H stretching region ($3,000\text{--}2,800\text{ cm}^{-1}$), peaks around $2,920\text{ cm}^{-1}$ and $2,850\text{ cm}^{-1}$ were observed, which were attributed to methyl and methylene groups, respectively. In T1, T2, and T3, slight shifts and a decrease in intensity were observed,

indicating modifications to the aliphatic structure of tempeh. In the Amide I and II region ($1,700\text{--}1,500\text{ cm}^{-1}$), peaks around $1,650\text{ cm}^{-1}$ (Amide I) and $1,550\text{ cm}^{-1}$ (Amide II) indicate C=O and N-H bonds from the protein.

The addition of seaweed caused shifts and changes in peak intensity, indicating interactions between tempeh protein and seaweed components, possibly forming new complexes or degrading peptide bonds. In the C-O stretching region ($1,250\text{--}1,000\text{ cm}^{-1}$), strong peaks in the $1,100\text{--}1,000\text{ cm}^{-1}$ range indicate ether (C-O-C) and alcohol (C-OH) groups. In T1 to T3, the peak intensity decreased, indicating chemical reactions between the carbohydrates in tempeh and seaweed components. All four samples shared the same main functional groups: O-H, C=O, and C-H groups. The differences were in the peak intensity, indicating varying amounts or compositions. These differences arose from the interactions between seaweed and tempeh, which altered the tempeh structure. Thus, tempeh with seaweed addition showed improved bioactive properties, such as higher antioxidant activity and phenolic content, despite slight reductions in some amino acid levels. This finding aligns with that of Arham *et al.* (2021), where a decreased intensity at $1,745$ and $1,543\text{ cm}^{-1}$ indicated protein degradation, showing reduced intensity in the amide range.

CONCLUSION

The addition of *E. spinosum* changed the amino acid composition of the tempeh. Increasing concentrations of *E. spinosum* led to higher levels of soluble proteins and antioxidant activity. Higher antioxidant activity is correlated with bioactive compounds such as phenolics, flavonoids, and carotenoid pigments, and is enhanced by isoflavones from soybean fermentation. Texture analysis showed that tempeh with added *E. spinosum* became softer, more elastic, easier to chew, and had better sensory quality. FTIR analysis confirmed the interactions between the seaweed components and the tempeh protein matrix, which modified the structure and enhanced the bioactive properties. Based on the results obtained in this study, the addition of 30% *E. spinosum* (T3) was found to be the optimal concentration for tempeh.

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