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Development of chickpea tempeh using *Rhizopus oryzae* for dysphagia diet: Effect of fermentation time and heat treatment

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ABSTRACT

Patients with dysphagia must manage their health with an appropriate diet. Chickpea tempeh is a nutritious source of carbohydrates and proteins, but its texture modification is necessary to suit varying severities of dysphagia, according to the International Dysphagia Diet Standardization Initiative (IDDSI) Framework. This study assessed the effects of fermentation time and heat treatment for development of tempeh classified as IDDSI level 6. The physicochemical and structural properties of tempeh were investigated, with a focus on biological changes during fermentation. Tempeh was prepared by grinding chickpeas to particle size of 3.50 ± 0.22 mm and fermenting for 24, 36, 48, 60, and 72 h using *Rhizopus oryzae*. During fermentation, the biological properties of tempeh changed, resulting in more white mold mycelia (cake) and the release of amylase and protease enzymes. Before cooking, tempeh exhibited increased hardness and brittleness by cake, as well as pH, total soluble solids, and DPPH increased by enzymetic activity. Fungal enzymes and heat treatment (175 °C) changed the rough and aggregated microstructure and molecular structure of chickpeas, as confirmed by scanning electron microscopy and Fourier transform infrared spectroscopy. The texture of cooked tempeh became softer and less brittle by fermentation time, attributed to weakening of cake bonds and enzymatic softening of chickpeas. IDDSI Framework results classified 24 and 36 h fermentation as level 7, and 48, 60, and 72 h fermentation as level 6. Tempeh with modified texture by heat treatment and controlled fermentation time may contribute to the improved health of patients with dysphagia.

1. Introduction

The recent shift toward a sustainable food system demands more plant-based food ingredients (Mookerjee & Tanaka, 2023). Tempeh is a traditional Indonesian fermented food that is produced through solid-state fermentation (SSF) of soybeans, utilizing a mold starter known as *Rhizopus* spp. (Erkan, Gürler, Bilgin, Germec, & Turhan, 2020). SSF is a bioprocessing technology that helps achieve the desired nutritional value, texture, and flavor of various legumes (Sánchez-Magaña et al., 2014). During SSF of legumes with *Rhizopus* spp., the following biological changes occur: (1) beans are bound together by grown white mold mycelia to form a cake, and (2) mold enzymes, which degrade the carbohydrates and proteins in beans into simpler compounds, are produced (Handoyo & Morita, 2006; Wang et al., 2023). Tempeh is generally produced using soybeans; however, various attempts are being made to ferment it using different types of legumes (Erkan et al., 2020; Polanowska, Grygier, Kuligowski, Rudzińska, & Nowak, 2020). Chickpea (*Cicer arietinum* L.) is a widely consumed pulse and a rich source of carbohydrates and protein. Chickpea starch has a low glycemic index due to high amylose content and resistant starch (Kaur & Prasad, 2021). The main proteins, globulin and albumin, have high bioavailability and low allergenicity compared to soybean protein (Boukid, 2021; Wang et al., 2021). However, chickpea has several undesirable attributes, such as a long cooking time and the presence of enzyme inhibitors and phytates (Angulo-Bejarano et al., 2008). Various methods, including milling, soaking, fermentation, germination, and thermal processing, can improve chickpea utilization (Kaur & Prasad, 2021). Several studies have investigated chickpea tempeh preparation via SSF with *Rhizopus* spp. (Angulo-Bejarano et al., 2008; Reyes-Moreno, Cuevas-Rodríguez, Milán-Carrillo, Cárdenas-Valenzuela, & Barrón-Hoyos, 2004). Although these studies evaluated the physicochemical and nutritional properties of chickpea tempeh flour, they did not comprehensively examine the structural and textural properties resulting from SSF-induced biological changes. To increase its consumption and utilization, tempeh must be developed into various products as sustainable, health-promoting foods gain popularity worldwide.

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Dysphagia refers to swallowing difficulties emanating from oropharyngeal or esophageal problems (Alagiakrishnan, Bhanji, & Kurian, 2013). Dysphagia severity in older individuals worsens due to tooth loss and reduced tongue muscle strength, potentially leading to malnutrition, aspiration pneumonia, and choking (Liu et al., 2023; Suebsaen, Suksatit, Kanha, & Laokuldilok, 2019). Texture-modified food (TMF) is normally prescribed to alleviate health risks in patients with dysphagia by enabling easy and safe consumption (Zarim, Zainul Abidin, & Ariffin, 2021). Particle size reduction (chopping, blending, and mincing) and hydrocolloid addition are two methods of obtaining dysphagia foods with an appropriate and consistent texture (Peh, Lim, Tha Goh, & Dharmawan, 2022; Pematilleke, Kaur, Rai Wai, Adhikari, & Torley, 2021). Hydrocolloids, the main strategy in TMF, are important food additives that act as thickeners and gelling agents, modifying food sensory properties. However, consumers are increasingly wary of food additives (Bolek, 2020; Li & Nie, 2016). The extent of texture-modification depends on the patient's severity of dysphagia (Pematilleke, Kaur, Adhikari, & Torley, 2022a). The International Dysphagia Diet Standardization Initiative (IDDSI) is the most recent guideline for categorizing texture modification in foods formulated for patients with dysphagia (Pematilleke, Kaur, Adhikari, & Torley, 2022b). The IDDSI framework comprises a continuum of eight levels (0–7) and provides standardized terminology and testing methods for TMFs and drink thickness according to dysphagia severity (IDDSI, 2019). IDDSI level 7 represents the "easy to chew" category, which is characterized by everyday foods with age-appropriate soft and tender textures. IDDSI level 6 denotes the "soft and bite-sized" category, wherein the recommended food piece size for adults should not exceed 15 mm to minimize the risk of choking. Dysphagia foods frequently lack carbohydrates and proteins due to nutritional dilution for texture modification, resulting in undernourished patients with a compromised immune system (Lou, Bilbao-Sainz, Wood, & Rubinsky, 2023). Therefore, it is crucial to explore alternative texture modification methods that do not use hydrocolloids-based nutritional dilution when developing TMF.

Chickpea tempeh is a potentially nutritious food for patients with dysphagia. Tempeh is generally prepared by fermenting and cooking the legume in its original form, and preliminary experimentation (Table S1) confirmed cooked tempeh's classification as an IDDSI level 7 dysphagia food. However, before providing chickpea tempeh to patients with different severities of dysphagia, texture modification is necessary. Therefore, this study aimed to prepare IDDSI level 6 chickpea tempeh by varying fermentation time with *Rhizopus oryzae* and using ground chickpeas with uniform, small-sized particle sizes. We investigated the structural and textural characteristics of chickpea tempeh, as a dysphagia food, based on biological changes in *R. oryzae* during fermentation and heat treatment.

2. Materials and methods

2.1. Materials

Commercially imported chickpeas (*Cicer arietinum* L.) cultivated in Canada were purchased from a local food store. They were fermented using *R. oryzae* starter powder (Cultures for Health, Morrisville, NC, USA). Brown rice vinegar (Ottogi Crop., Anyang, Korea), soluble starch, ethanol (Duksan Co., Ltd., Ansan, Korea), milk casein, Folin–Ciocalteu phenol reagent (Junsei Chemical Co., Ltd., Tokyo, Japan), 2,2-diphenyl-1-picryl hydrazyl (DPPH), 3,5-dinitrosalicylic acid (DNS), maltose, tyrosine (Sigma-Aldrich, St Louis, MO, USA), and trichloroacetic acid (TCA; Wako, Osaka, Japan) were used. All chemicals were reagent grade or purer.

2.2. Solid-state fermentation (SSF) of tempeh

Whole seeds of washed chickpeas (300 g) were soaked in 900 mL of distilled water (DW) at room temperature (25 °C) for 12 h and subsequently cooked in DW (1200 mL) at 98 ± 2 °C for 30 min. After draining and cooling to room temperature, the cooked chickpeas were ground to reduce their particle size from 13.98 ± 0.35 mm to 3.50 ± 0.22 mm using a blender at 13,000 rpm for 20 s. Based on 300 g of raw materials, ground chickpeas were thoroughly mixed with vinegar (pH 2.80) of 20 g, and inoculated with 3 g of tempeh starter. Vinegar is utilized to lower the pH and regulate the microbial composition of tempeh (Dahlan, Nambu, Putri, & Fukusaki, 2022). The resulting mixture (70 g) was packed in regular-sized perforated polyethylene bags, with spacing allowing for aerobic conditions, and subsequently molded to the following dimensions: length 120 mm × width 80 mm × height 15 mm. Tempeh was fermented at 33 ± 2 °C for 24, 36, 48, 60, and 72 h, following to the method described by Reyes-Moreno et al. (2004) and preliminary experiments.

2.3. Biological properties

The SSF-induced formation of white mold mycelia was visually evaluated. Enzyme activity was estimated using a modified method from Konkit and Kim (2016). Lyophilized tempeh flour (1 g) was suspended in 10 mL of DW for 30 min at room temperature with shaking, then centrifuged at 15,000 *g* and 4 °C for 10 min to prepare the enzyme extract. For amylase activity, 0.5 mL of enzyme extract was incubated with 0.5 mL of 1 % soluble starch at 37 °C for 5 min. After adding 0.5 mL of DNS, the mixture was incubated at 95 °C for 15 min, cooled, and diluted with 9 mL of DW. Absorbance was measured at 540 nm using a spectrometer (Evolution 201 UV–Visible Spectrophotometer, ThermoFisher Scientific Inc., Brooklyn, NY, USA). Maltose (1 mM) was used as a standard, and one unit of amylase activity was defined as the amount of enzyme required to release 1 μmol/min of reducing sugar. For protease activity, 1 mL of enzyme extract was added to 1 mL of 1 % casein and incubated at 37 °C for 30 min. After adding 2 mL of 10 % TCA to terminate the reaction, the mixture was filtered through Whatman filter paper No. 1. Test filtrate (1 mL), 2.5 mL of Na_2CO_3, and 0.5 mL of 50 % Folin–Ciocalteu phenol reagent were added to each tube. The resulting mixture was thoroughly mixed well and incubated at 37 °C for 30 min. Absorbance was read at 660 nm. Tyrosine (1 mM) was used as a standard, and one unit of protease activity was defined as the amount of enzyme required to release 1 μmol/min of tyrosine.

2.4. Physicochemical properties and DPPH assay

Color values (CIE LAB) were measured on the internal surface of tempeh using a colorimeter (CR-400, Minolta Co., Ltd., Osaka, Japan) calibrated with a standard white plate ($L^* = 83.83$, $a^* = 0.29$, and $b^* = 28.97$). L^*, a^*, and b^* values indicate lightness, redness, and yellowness, respectively. Tempeh was diluted 1:4 (*w/v*) with DW and ground using a hand blender for 1 min; subsequently, pH, total soluble solids (TSS, °Brix), and viscosity were analyzed. pH and TSS were measured using a pH meter (Orion 3 star, ThermoFisher Scientific Inc., Brooklyn, NY, USA) and refractometer (Master-a, Atago Co., Ltd., Tokyo, Japan), respectively. Viscosity was determined after running at 100 rpm for 1 min using rotational viscometer (DVIM, Brookfield Engineering, Middleborough, MA, USA) with a spindle #64. DPPH free radical scavenging activity was evaluated according to method of Alves Magro and de Castro (2020), with slight modifications. Briefly, 0.2 mL of tempeh ethanol extract of tempeh was incubated with 2 mL of 1 mM DPPH ethanol solution in the dark (25 °C, 30 min); thereafter, absorbance was measured at 518 nm using a spectrometer. DPPH radical scavenging activity (%) was calculated using the following formula: $([ABS_{control}-ABS_{sample}]/ABS_{control}) \times 100$, where $ABS_{control}$ and ABS_{sample} represent the absorbance of the control and sample, respectively.

2.5. Cooking conditions

Cooking conditions were based on the method of Aaslyng and Højer

(2021), with modifications. Before cooking, tempeh was cut into same-sized samples (length/width, 15 mm; height, 10 mm). Thereafter, the samples were cooked in an aluminum tray for 5 min (turned over once) using a convection oven (Bread oven, Kumbok Stock, Seoul, Korea) set at 175 °C. The cooked samples were cooled to 25 °C within 8 min and kept at room temperature for further analysis.

2.6. Changes in structural and textural characteristics

Changes in the structural and textural properties of tempeh by fermentation time and heat treatment were analyzed in terms of microstructure, molecular-level information, and texture profile.

2.6.1. Scanning electron microscopy (SEM)

The microstructure of tempeh was analyzed using a scanning electron microscope (SU8220, Hitachi Ltd., Tokyo, Japan). Lyophilized tempeh was attached to SEM sample holders and sprayed with platinum at 20 mA for 160 s under vacuum using an ion sputter coater (E-1030, Hitachi Ltd., Tokyo, Japan). Images were taken at 150× magnification.

2.6.2. Fourier transform infrared (FT–IR) spectroscopy

FT–IR spectroscopy was performed using an FT–IR spectrometer (Perkin Elmer Inc., Waltham, MA, USA) equipped with a Quest attenuated total reflectance. The spectra were recorded by scanning lyophilized tempeh flour at wavelengths of 4000 to 400 cm^{-1}, a scan speed of 0.2 cm/s, and a resolution of 4 cm^{-1}.

2.6.3. Texture profile analysis (TPA)

TPA was performed on same-sized tempeh samples that was 15 mm × 15 mm × 10 mm (length×width×height) using a rheometer (Compac-II, Sunscientific Co., Ltd., Tokyo, Japan) with a 50 mm cylindrical probe. The testing conditions were as follows: pre-test speed of 2 mm/s, test and post-test speeds of 1 mm/s, compression distance of 2 mm, clearance of 7 mm, and loadcell of 2 Kgf. The parameters assessed using the two-bite compression test included hardness, springiness, cohesiveness, gumminess, and brittleness.

2.7. International dysphagia diet standardization initiative (IDDSI) tests

Cooked tempeh samples (length/width, 15 mm; height, 8 mm) were categorized into IDDSI levels 6 (soft and bite-sized) and 7 (easy to chew) (IDDSI, 2019) based on the preliminary results. Samples were evaluated using the fork/spoon pressure and fork separation test according to the IDDSI testing methods. Pressure (approximately 17 kPa) is applied until the thumb nail blanches to white, thus mimicking tongue force during food swallowing. To determine the IDDSI level, sample behavior was observed and compared using IDDSI protocols.

2.8. Statistical analysis

The experiments were replicated at least three times and all data are presented as the mean ± standard deviation. Statistical analyses were performed using SPSS software (version 26, IBM SPSS Statistics Inc., Chicago, IL, USA). The results were analyzed using one-way analysis of variance with Duncan's multiple-range test. Changes in TPA results before/after cooking were compared using Student's *t*-test. Statistical significance was set at $p < 0.05$.

3. Results and discussion

3.1. Biological properties

Biological changes in chickpea tempeh in terms of mold mycelium formation and enzyme production were analyzed during SSF with *R. oryzae*. As shown in Fig. 1, white mycelium formed on the interior and exterior surfaces of tempeh via fermentation. Fungal species such as *R. orzyae* are characterized by sexual reproduction via zygospores, asexual reproduction by formation of sporangiospores, and in most species, by non-septate hyphae (Londoño-Hernández et al., 2017). White mycelium formation increased on ground chickpeas with increasing fermentation time, eventually covering their entire surface at 60 h of fermentation. Owing to limited airflow in the packaging during SSF, *Rhizopus* spp. sporangiospores tend to disperse and grow into a dense white mycelium that binds the legume together to form a compact cake (Ahnan-Winarno, Cordeiro, Winarno, Gibbons, & Xiao, 2021). Chickpea tempeh fermented for 96 h was over-fermented, exhibiting a yellow, caramel-like color and emitting an ammonium odor (Fig. S1). Liu et al. (2023) reported that tempeh fermented with *R. oligosporus* covered the surface of soybeans with mycelium after 2 days of fermentation, and the color and odor of tempeh changed from 72 h of fermentation. These results suggest that before over-fermentation, tempeh exerts a different binding force on ground chickpeas, influenced by the white cake formed during fermentation.

Figure 2 displays the enzyme activity in chickpea tempeh according to fermentation time. During fermentation, *R. oryzae* primarily utilizes glucose as a carbon source and urea as a nitrogen source, leading to the production of amylase and protease as hydrolases (Londoño-Hernández et al., 2017). The results indicate that amylase and protease activities significantly increased with fermentation time, peaking at 36 h of fermentation. Huang et al. (2019) reported that, unlike in tempeh flour, enzyme activity was not detected in non-fermented soybean flour. The lowest amylase activity value (11.97 U/mL) was detected at 24 h of fermentation, and amylase activity gradually decreased after 36 h of fermentation (Fig. 2A). Protease activity also exhibited a similar tendency, with the lowest level observed after 24 h of fermentation at 7.44 U/mL (Fig. 2B). Prolonged incubation results in decreased enzyme production consequent to nutrient reduction in the substrate as well as amylase and protease release (Benabda, M'Hir, Kasmi, Mnif, & Hamdi, 2019). Mold fermentation increased the protein content of chickpea tempeh compared with those of raw chickpea, and longer fermentation time positively affected the nutritional quality of tempeh (Table S2). According to Drabo, Savadogo, and Raes (2023), fermentation with *R. oryzae* increased the carbohydrate and protein contents and removed phytate, enhancing the nutritional value of tempeh. In SSF of chickpea tempeh, enzyme production by *R. oryzae* was highly influenced by fermentation time. Various enzymes are produced during fermentation, and they degrade carbohydrates and proteins into simpler compounds, thus altering the nutritional and physicochemical properties of soy products (Wang et al., 2023).



Fig. 1. Visual appearance of tempeh with different fermentation times.

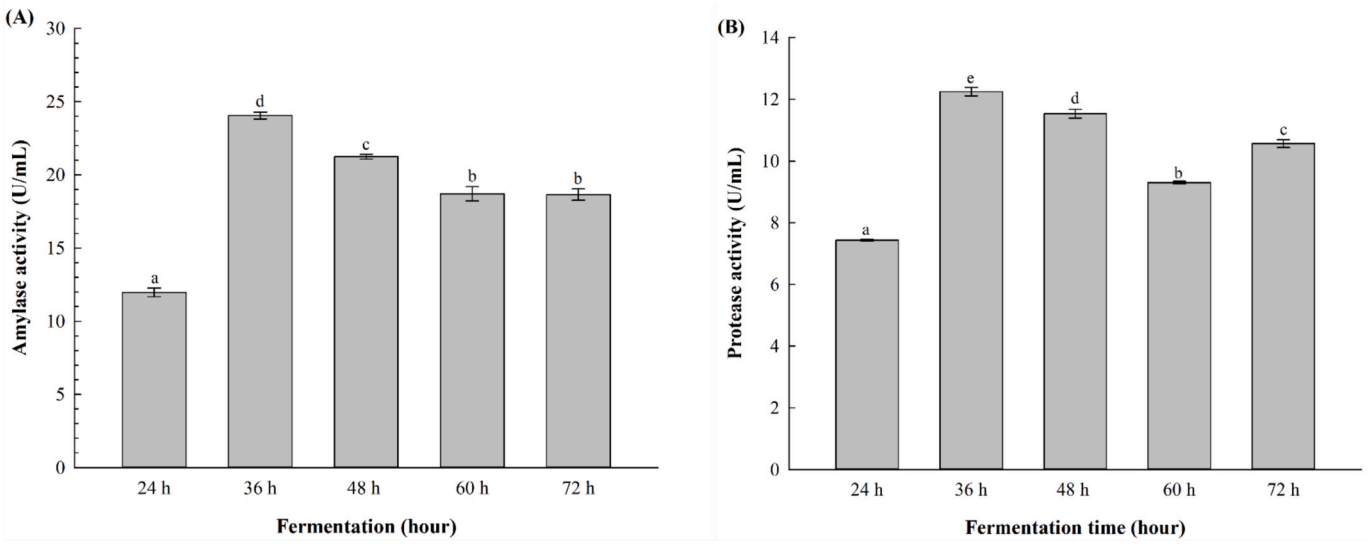


Fig. 2. Amylase activity (A) and protease activity (B) of tempeh with different fermentation times. Data are expressed as the mean ± SD ($n = 3$). Different letters in each bar represent significant differences (*$p < 0.05$).

3.2. Physicochemical properties

The effects of fermentation time-induced biological changes on the physicochemical properties of chickpea tempeh are shown in Table 1. White mycelium caused by *R. oryzae* gradually covered the surface of ground chickpeas during SSF (Fig. 1). Depending on fermentation time, the internal color of tempeh tended to increase in L^* value and decrease in b^* value, implying that the beige color of the chickpeas has changed to white as the white mycelium becomes more pronounced. The L^* value was significantly higher for tempeh samples fermented for 48 to 72 h, and while the b^* value was lower after 72 h of tempeh fermentation than for 48 and 60 h of fermentation ($p < 0.05$). The a^* value of tempeh displayed no significant difference during fermentation. Previous research has reported contrasting results, indicating a decrease in the L^* value and an increase in the b^* value of tempeh color during fermentation with soybeans and *R. oligosporus* (Handoyo & Morita, 2006). The color of tempeh considered to be influenced by the type of *Rhizopus* spp. and legumes used in SFF. The pH of chickpea tempeh increased from 5.38 to 6.15 during SSF ($p < 0.05$). Moa et al. (2013) reported that the use of *Rhizopus* starter during fungal fermentation resulted in a gradual increase in tempeh pH. This shift in pH is attributable to effective protein hydrolysis by the mold during fermentation, resulting in the release of amines and ammonia and subsequent elevation in pH (Liu, Guo, Liu, Yolandani and H., & Ashokkumar, M., 2023) As shown in Fig. 2B, higher levels of protease activity by *R. oryzae* were detected from 36 h of fermentation, confirming an increased in tempeh pH. The TSS of chickpea tempeh increased with fermentation time. According to Kizzie-Hayford et al. (2021), the °Brix of tiger nut increased during storage owing to α-amylase activity. Amylase activity was stimulated during SSF of tempeh (Fig. 2A), thus affecting the TSS owing to the hydrolysis of chickpea polysaccharides into monosaccharides. The viscosity of the tempeh suspension significantly increased with fermentation time. The sample fermented for 72 h exhibited more than two-fold greater viscosity than those fermented for shorter periods. The white mycelium produced by *R. oryzae* potentially increases the viscosity of the tempeh suspension (Riley, Tucker, Paul, & Thomas, 2000). Fermentation also supported enzymatic hydrolysis of starch, resulting in a lager soluble starch fraction, contributing to the increased viscosity (Pranoto, Angrahini, & Efendi, 2013). The most commonly used assay for evaluating the antioxidant activity of food and plant extracts is the DPPH radical scavenging method (Tan & Lim, 2015). DPPH radical scavenging activity of chickpea tempeh increased from 6.44 % to 14.74 % as fermentation time increased ($p < 0.05$). Janarny and Gunathilake (2020) reported that the total antioxidant capacity, including DPPH, increased by the SSF process using *R. oryzae*. Several active peptides and amino acids were produced via chickpea protein hydrolysis by proteinases after fermentation and may play an important role in improving the antioxidative activity of chickpeas (Li & Wang, 2021). As SSF progressed, high protease activity was achieved in chickpea, ultimately improving the antioxidant capacity of tempeh.

3.3. Scanning electron microscopy (SEM)

Morphological differences in chickpea tempeh according to

Table 1
Physicochemical properties of tempeh with different fermentation times.

Fermentation time (hour)	Color values			pH	Total soluble solids (°Brix)	Viscosity (cP)	DPPH radical scavenging (%)
	L^*	a^*	b^*				
24	79.41 ± 0.94^{a}	−0.41 ± 0.13^{b}	36.26 ± 1.10^{d}	5.38 ± 0.03^{a}	3.00 ± 0.00^{a}	148.00 ± 3.46^{a}	6.44 ± 0.31^{a}
36	81.26 ± 0.74^{b}	−0.45 ± 0.15^{ab}	34.44 ± 0.42^{c}	5.51 ± 0.01^{b}	3.27 ± 0.06^{b}	168.00 ± 6.00^{b}	8.44 ± 0.27^{b}
48	83.10 ± 0.57^{c}	−0.54 ± 0.19^{ab}	32.52 ± 1.07^{b}	5.60 ± 0.05^{c}	3.50 ± 0.10^{c}	196.00 ± 3.46^{c}	10.74 ± 0.57^{c}
60	83.59 ± 0.48^{c}	−0.54 ± 0.10^{ab}	31.33 ± 0.88^{b}	5.99 ± 0.02^{d}	3.63 ± 0.06^{cd}	264.00 ± 15.87^{d}	12.50 ± 0.27^{d}
72	83.96 ± 0.52^{c}	−0.65 ± 0.14^{a}	28.78 ± 1.16^{a}	6.15 ± 0.01^{e}	3.67 ± 0.12^{d}	340.00 ± 3.46^{e}	14.74 ± 0.53^{e}

Data are expressed as the mean ± SD (n = 3, color values; $n = 5$); Different letters within a column represent significant differences (*$p < 0.05$); L^*: lightness; a^*: redness (positive value) and greenness (negative value); b^*: yellowness (positive value) and blueness (negative value).

fermentation time and heat treatment were analyzed. The surface microstructure of tempeh was examined using SEM (Fig. 3). In the microstructure of chickpea, starch granules vary in shape from ovoid to spherical, and the discernible globular or irregular particles attached to or located between the starch granules comprise broken protein bodies or fragments of the protein matrix (Ma et al., 2011). In the tempeh fermented 24 and 36 h, spherical and irregular particles were clearly observed, and the surfaces of partly particles were loose and dispersed. The lengths of clearly observable chickpea particles fermented for 24 and 36 h ranged from 1.30 to 1.91 mm and from 0.88 to 1.63 mm, respectively. According to Wang et al. (2023), the microstructure of unfermented soybean meal revealed starch granules and a protein matrix structures with well-defined boundaries. As the fermentation time increased, the tempeh granules became rougher and appeared to have an aggregated surface. Clearly spherical particles were not observed from 48 h of fermentation. Amylase and protease not only increase the amount of essential components but also degrade the substrate's cell structures (Handoyo & Morita, 2006). The epidermal structure of chickpea tempeh particles was damaged during SSF, possibly because of carbohydrate and protein hydrolysis induced by fungal enzymes released from *R. oryzae*, shown in Fig. 2. Akpabli-Tsigbe, Osabutey, Mintah, Tano-Debrah, and Ma (2023) reported similar results wherein enzymes produced during SSF played a role in changing the surface of soybeans into agglomerated structure. It was confirmed that the pH and TSS values of tempeh increased during fermentation by enzyme activity (Table 1), and therefore, hydrolytic enzymes were involved in modifying both the physicochemical as well as structural properties of tempeh. The microstructures of heat-treated tempeh exhibited a different morphology from that of unheated tempeh. The heat-treated tempeh granules comprised a more irregular, amorphous, and roughened surface structure. In tempeh fermented for 24 h, clear spherical particles with lengths of 1.00 to 1.79 mm were partially observed even after cooking, and heat-treated tempeh displayed an increase in the degree of surface agglomeration with fermentation time. This phenomenon may emanate from heat treatment of the starch and protein mixture, resulting in a homogenous network comprising cross-linked starch and protein molecules (Ma et al., 2011). The ground chickpeas allowed the interaction of starch granules with water during tempeh preparation, which along with heat treatment increased the amount of disrupted starch granules, thus enhancing their pre-gelatinization (Silvestre-De-León, Espinosa-Ramírez, Heredia-Olea, Pérez-Carrillo, & Serna-Saldívar, 2020). At high temperature, protein aggregation and folding could be observed owing to interactions between protein granules in tempeh, exhibiting consistency with the results of previous studies (Wang et al., 2023).

3.4. Fourier transform infrared (FT–IR) spectroscopy

FT–IR spectroscopy is useful for identifying functional groups in organic compounds (Silviani et al., 2023). The FT–IR spectra of fermented and heat-treated chickpea tempeh are illustrated in Fig. 4A. The strong and broad absorption peak in the 3300 cm^{-1} region was characteristic of polysaccharides, indicating the absorption of stretching vibrations of O—H bonds (Ninčević Grassino et al., 2020). Differences in the hydrogen bonding-related 3300 cm^{-1} peak according to fermentation time and heat treatment were observed. The tempeh bands before cooking were 3285–3265 cm^{-1}, while those after cooking were 3281–3265 cm^{-1}. The absorption wavelength tended to decrease with fermentation time and heat treatment, suggesting that more hydrogen bonds were formed and interacted with other groups (Arfat, Benjakul, Prodpran, & Osako, 2014). The absorption band indicated the presence of polysaccharide hydroxyl groups, which constitute most of the cell walls in the mycelium (Hamza, Mandari, & Santhosh Kumar, 2023). The 2929 to 2923 cm^{-1} peak was related to lipids (Sofi et al., 2023). Bands characteristic of protein, this is, amide I (~1635 cm^{-1}), amide II (~1546 cm^{-1}), and amide III (~1246 cm^{-1}), were explicitly observed (Zha, Gao, Rao, & Chen, 2021), and these bands shifted to other bands or disappeared upon fermentation and heat treatment. The amide I bands of tempeh before cooking were 1633–1621 cm^{-1}, while those after cooking were 1625–1621 cm^{-1}. As fermentation time and heat treatment increased, the amide I band yielded lower values, and the observed shifts were attributed to modification of the C═O and C═N bonds in the secondary structures of proteins (Aref & Salem, 2020). Fermentation may be responsible for to lower amide I peak intensity and narrower peak area, which reduce the cross-linking of peptide fragments (Chen et al., 2022). The peak around 1635 cm^{-1} may be largely attributed to the random coil in the protein secondary structure (He, Liu, Zhou, & Huang, 2018). Yang, Li, Lin, Zhang, and Chen (2017) reported that a lower random coil content is associated with higher DPPH activity. The amide II band represents NH flexural and CN tensile vibrations (Gómez-Mascaraque, Tordera, Fabra, Martínez-Sanz, & Lopez-Rubio, 2019). The intensity of the amide II peak gradually decreased with fermentation time, and the bands disappeared from 60 h of fermentation before cooking and from 48 h of fermentation after cooking. This indicates the absence of amide II owing to fermentation and heat treatment. In the amide III region, the bands revealed increasing wave number with fermentation and heat treatment. Chen et al. (2022) reported that the vibrations of the bonds in the amide III region potentially suggests alterations in the N—H and C—N bonds resulting from *endo*- and *exo*-enzymes. Characteristic peaks at 1027 cm^{-1} arising from starch corresponded to the considerable C–C–O or C–C–OH bending vibrations (Kim, Kim, Lim, & Moon, 2024). In addition, the peaks observed near 760–520 cm^{-1} provided evidence for the presence of glycosidic groups

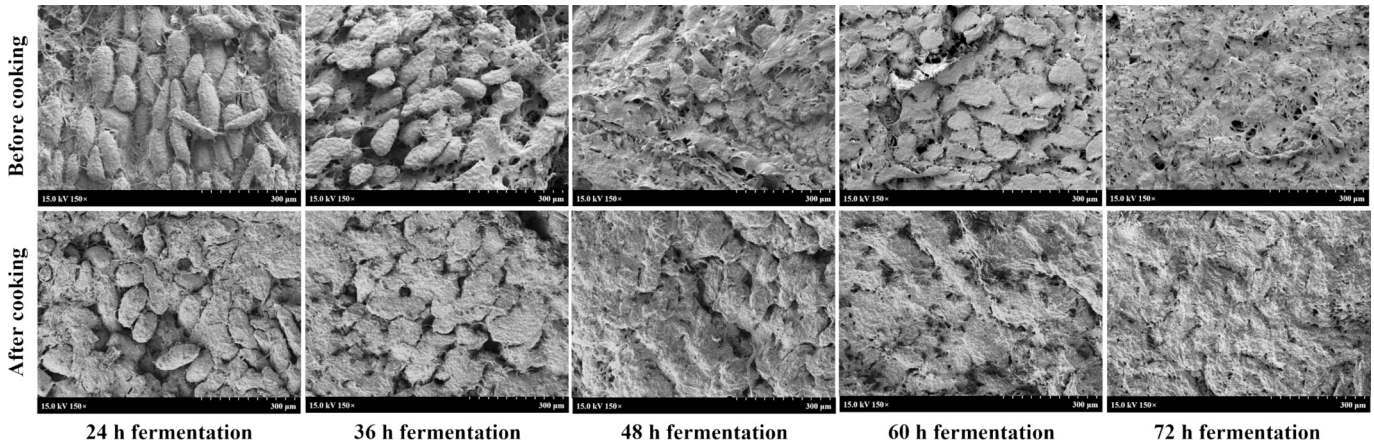


Fig. 3. Scanning electron microscopy (magnification 150×) of tempeh and cooked tempeh with different fermentation times.

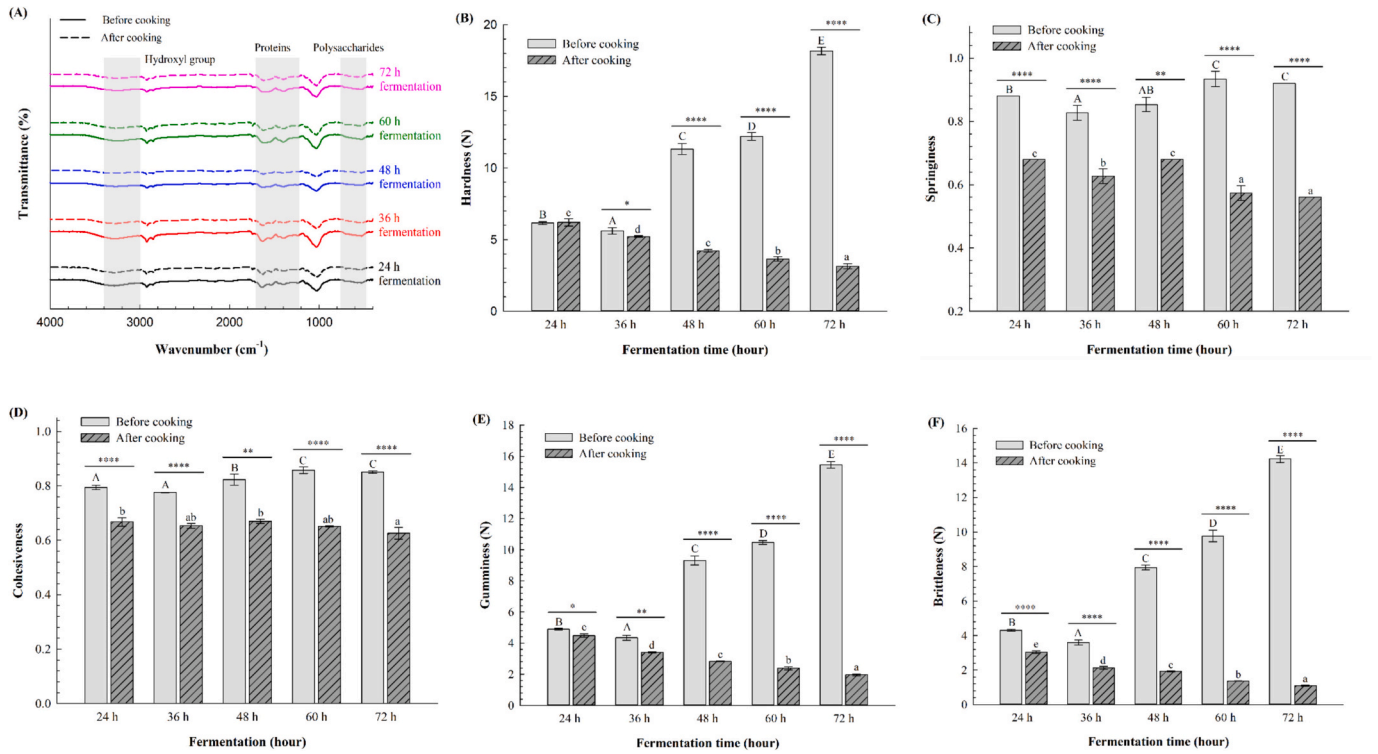


Fig. 4. FT-IR spectrum (A), hardness (B), springiness (C), cohesiveness (D), gumminess (E), and brittleness (F) of tempeh and cooked tempeh with different fermentation times. Data are expressed as the mean ± SD (n = 3). Different letters in each bar represent significant differences (*p < 0.05, **p < 0.01, and ****p < 0.0001).

between glycosyl groups (Yalmanci, İspirli, & Dertli, 2022). The magnitude of these peaks decreased with fermentation time, possibly because of starch hydrolysis by amylases (Sofi et al., 2023). Fermentation with *R. oryzae* and heat treatment can cause structural modifications in chickpea, leading to the formation of new protein-starch and protein-protein interactions.

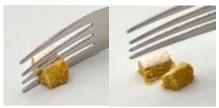
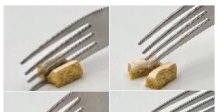
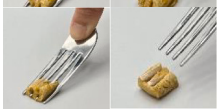
3.5. Texture profile analysis (TPA)

TPA was applied to characterize the texture of chickpea tempeh according to fermentation time and heat treatment. Texture characterization is important for food quality and directly related to consumer preference (Xu et al., 2021). The textural properties of tempeh, such as hardness, springiness, cohesiveness, gumminess, and brittleness, were altered by increasing fermentation time and cooking. Before cooking, hardness (Fig. 4B) rapidly increased from 48 h of fermentation as fermentation time increased; however, after cooking, it significantly decreased. Tempeh fermented for 24 h showed no difference in hardness according to heat treatment; nevertheless, after 36 h of fermentation, hardness sharply decreased ($p < 0.0001$). The springiness value represents the recovery ability of a sample from the initial deformation (Karki, Oey, Bremer, Leong, & Silcock, 2023). The springiness (Fig. 4C) of the samples fermented for 60 and 72 h was highest before cooking and lowest after cooking ($p < 0.05$). Springiness before/after cooking decreased significantly in all samples. Cohesiveness (Fig. 4D) increased from 48 h of fermentation before cooking ($p < 0.05$), and no difference was noted between samples after cooking. Heat treatment significantly reduced the cohesiveness of tempeh. Gumminess, the force required to disintegrate a semisolid sample into a steady state for swallowing, is calculated by multiplying the hardness value with the cohesiveness value (Martinez, Salmerón, Guillén, & Casas, 2004). Gumminess (Fig. 4E) exhibited a similar overall trend to that of hardness. Brittleness (Fig. 4F) rapidly increased from 48 h of fermentation before cooking, and significantly decreased with fermentation time in heat-treated tempeh. Brittleness tended to decrease before and after cooking ($p < 0.0001$). As fermentation progressed, the structure of tempeh became hard and brittle, which is considered to be influenced by the continued formation of white mycelium as indicated by the visual appearance (Fig. 1) and color values (Table 1). *Rhizopus* spp. sporangiospores tend to disperse and grow into a dense white mycelium that binds the soybean together to form a compact cake (Romulo & Surya, 2021). Handoyo and Morita (2006) reported similar results indicating that the network between soybean grains of tempeh became stronger after 48 h of fermentation, by which time the mycelium had grown abundantly. After cooking, tempeh appeared to have a softer and less brittle texture depending on fermentation time, suggesting that heat treatment at 175 °C weakened the compact bond of the cake formed during fermentation. Mycelium generated by fungal fermentation after pyrolysis exhibits reduced fibrous networks, mycelium diameters, and cell wall thickness (Jones et al., 2018). On the other hand, fungal enzymes released from *R. oryzae* hydrolyzed macromolecules in chickpea (Fig. 2), softening the texture of chickpea tempeh. Although TPA is widely available and convenient for rapid food texture evaluations, it may not be reliable enough to determine the feasibility of dysphagia diets when applied alone (Nishinari, Kohyama, Kumagai, Funami, & Bourne, 2013). Dick, Bhandari, and Prakash (2021) reported a hardness of 1.73–6.90 N in modified-texture cooked beef pastes classified as IDDSI level 6. The hardness values of cooked tempeh ranged from 3.14 to 6.20 N, which can be considered suitable for dysphagia foods.

3.6. International dysphagia diet standardization initiative (IDDSI) tests

The IDDSI framework is a global standard and testing method for evaluating foods intended for patients with dysphagia (IDDSI, 2019). Although tempeh is a considerably nutritious food, texture modification is important when providing tempeh to patients with varying dysphagia severities. The IDDSI test results of cooked chickpea tempeh according to fermentation time are shown in Table 2. A level 6 dysphagia diet is

Table 2
The IDDSI tests of cooked tempeh with different fermentation times.

Fermentation time (hour)	IDDSI tests			
	Fork pressure test	Spoon pressure test	Fork separation test	Comments
24				Level 7 (Easy to chew)
36				Level 7 (Easy to chew)
48				Level 6 (Soft and bite-sized)
60				Level 6 (Soft and bite-sized)
72				Level 6 (Soft and bite-sized)

pressed with the base of a fork/spoon with enough pressure that the thumb nail blanches to white; the food sample should be squashed until it breaks apart without returning to its original shape when the fork/spoon pressure is released (Cichero & Correspondence, 2020). In all samples with length×width×height of 15 mm × 15 mm × 10 mm, the thumb nail turned white when pressure was applied and did not return to its original shape/color when the pressure was released. The pressure (approximately 17 kPa) that makes the thumb nail blanch reflects the force of the tongue during swallowing (Steele et al., 2015). In addition, the fork/spoon pressure test was used to determine the samples' degree of brittleness under the pressure of a fork/spoon. The recommended piece size of level 6 dysphagia food for adults should <15 mm to minimize the risk of choking (IDDSI, 2019), and this criterion can also be used to classify level 6 and 7 foods. Tempeh fermented for 24 and 36 h could easily be disintegrated into smaller pieces by applying pressure using a fork/spoon, and pressured samples might have exceeded 15 mm. According to Min et al. (2023), gel broken into small pieces using a fork/spoon was classified as level 7 because gel piece size was not restricted. Therefore, the particle size (13.98 ± 0.35 mm) of whole chickpeas before SSF was smaller and uniformly crushed to 3.50 ± 0.22 mm. From 48 h of fermentation, tempeh was evidently in a form in which crushed chickpeas were agglomerated and compressed without breaking into particles. This is presumably because of the effect of increasing fermentation time on ground-chickpea softening by the hydrolytic action of fungal enzymes as well as heat treatment-induced starch gelatinization and protein aggregation. In all samples, food was easily broken apart with the side of the fork during the fork separation test; therefore, chickpea tempeh was considered a dysphagia food with a soft/tender texture according to the IDDSI (2019). Based on three tests, tempeh fermented for 48, 60, and 72 h corresponded to level 6 of the IDDSI framework, which is highly desirable for a dysphagia diet.

4. Conclusion

In this work, chickpea tempeh was developed as a dysphagia food by varying fermentation time and heat treatment to increase its sustainable and health-promoting use. Cooked tempeh that had been fermented for 48 to 72 h was classified as level 6 compliant with the IDDSI framework. The texture of cooked tempeh was modified by incorporating ground chickpeas and adjusting the fermentation time, without the addition of hydrocolloids or the dilution of nutritional. The process resulted in a texture-modified tempeh that is suitable for consumption by patients with dysphagia. In addition, the structural and textural properties of tempeh were analyzed based on the biological changes that occur during SSF, and heat treatment. On fermenting with *R. oryzae*, the fungal mycelium elicited tight bonds between ground chickpea particles, generating white cake, and fungal enzymes softened the ground chickpeas by hydrolyzing major macromolecules, such as carbohydrates and proteins. Heat treatment caused the gelatinization and aggregation of hydrolyzed starch and protein, respectively. Therefore, the texture of cooked tempeh required less chewing ability and became less brittle as fermentation time increased. These biological changes during SSF and heat treatment caused chickpea tempeh to adopt a more desirable texture for patients with dysphagia. Nevertheless, merely controlling the fermentation time presents challenges in supplying tempeh to patients with more severe dysphagia. Future research will focus on development of texture-modified tempeh to IDDSI levels 4 and 5, utilizing protein-based thickeners in conjunction with 3D food printing technology.

CRediT authorship contribution statement

Jung Soo Kim: Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Jiyoon Kim:** Writing – review & editing, Visualization, Validation, Methodology, Data curation. **Soo Hyun Kim:** Writing – original draft, Methodology, Investigation. **Kwang-Deog Moon:** Validation, Supervision, Resources, Funding acquisition.

Declaration of competing interest

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ifset.2025.103940.

Data availability

Data will be made available on request.

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