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A review on health benefits and processing of *tempeh* with outlines on its functional microbes

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ABSTRACT

Tempeh is a nutritious fermented legume food made from soybeans and commonly consumed in Southeast Asia especially in Indonesia and Malaysia. The fermentation process involves growth of mold, *Rhizopus* spp. which transforms soybeans into a white firm cake-like product as enzymes break down complex nutrients in soybeans into simpler forms making the high protein and nutrient rich food more bioavailable. Soybeans fermentation also leads to the production of metabolites such as organic acids, antioxidants and antimicrobial compounds which increase the vitamins content, enhance the nutritional value and shelf life of *tempeh*. Consumption of *tempeh* has been linked to various health advantages including antidiabetic effects, cholesterol-lowering properties, improved cognitive function, antitumor and anticancer properties, anti-aging effects and improved gut health as well as reduced risk of cardiovascular disease. This paper summarises useful information on *tempeh* which includes health benefits, nutrition and functional microbes isolated. It includes information on processing and substrates to provide more avenues to novel and sustainable food product development as *tempeh* offers an excellent alternative to plant-based protein source.

1. Introduction

Soybean is a type of legume that originated in East Asia, one of the most essential crops globally today. Currently, the world's largest soybean producers to-date are Brazil, followed by the United States and Argentina. Production of soybeans has risen year after year to meet the high demand for soybean oil as a feedstock for biodiesel production (USDA 2022). Today, soybeans are a valuable source of dietary protein and oil in the marketplace. Soy protein is increasingly recognised for its nutraceutical properties as it is high in essential amino acids, folic acid, isoflavones (naturally occurring compounds found in plant-based foods that mimic the functions of the hormone estrogen, such as genistein, daidzein, and daidzein), saponins (amphiphilic compounds that give emulsifying properties) (Shi et al., 2004), phytic acids, trypsin inhibitors, and low amount of saturated fat making it an ideal food for a healthy diet (Isanga and Zhang 2008). It is a versatile food ingredient that can be used to make a wide range of fermented soy products such as *tempeh*, miso, natto, tofu, and soy sauce (Liu et al., 2022). The fermentation process enhances the texture and flavor of soybeans, as well as increases its nutritional value which is beneficial to human health (Granito et al., 2002). It also brings about significant biochemical changes through the action of microorganisms or enzymes (Campbell-Platt 1994). *Tempeh* (or *tempe*) is unique being the only fermented soy product that did not originate in China or Japan. It is originated from Indonesia and is a well-known traditional fermented food popularly consumed in Malaysia. *Tempeh* serves as an alternative nutrient-dense source of protein at an affordable price hence has become a staple food for the locals (Astuti et al., 2000).

According to Nout & Rombouts (1990) and Owens (2014), *tempeh* is produced via a two-step fermentation process, which involves a soaking process and solid-state fermentation using various strains of mycelium fungus, e.g. the *Rhizopus* spp. (*R. oligosporus, R .oryzae,* and *R. stolonifera)*. After the fermentation process, the soybeans are held together by a dense white cottony mycelium forming a compact cake (Hachmeister and Fung 1993). Generally, *tempeh* is consumed in the form of deep-fried slices, used in soups, or cooked with chili and spices in

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coconut milk and rarely consumed fresh. Historically, *tempeh* was perceived as a protein source for the lower classes, while animal protein was considered a food for the upper class. Today, *tempeh* is widely consumed by people and has gained greater acceptance as well as increasingly accessible in supermarkets and upscale restaurants (Owens 2014). It is most suitable for vegetarians as meat replacer as it is a good source of non-animal protein. It is high in protein, prebiotics, and a wide array of vitamins and minerals making it a full-fledge meat substitute (Cabello-Olmo et al., 2019; Gunawan-Puteri et al., 2015).

2. Health benefits of *tempeh* consumption

2.1. Anti-diabetes effects

The diverse array of health benefits demonstrated in Fig. 1 underscores the importance of incorporating tempeh in our diet to promote overall well-being such as anti-diabetes effects. Diabetes mellitus stands as a chronic metabolic disorder characterised by high levels of blood glucose arising from either an insufficient production of insulin or a reduced sensitivity to insulin which over time can result in severe harm to human (Sun et al., 2020). According to World Health Organization (WHO), approximately 422 million people worldwide are affected by diabetes, with the majority residing in low- and middle-income countries and about 1.5 million deaths are directly attributed to diabetes annually (WHO 2023). There has been a gradual increase in both the incidence and prevalence of diabetes over the past few decades, thus raising awareness on this issue is crucial.

A major factor in the effort to lower the risk of type 2 diabetes (T2DM) is being mindful of our dietary choices and habits developed during our meals. *Tempeh* is undeniably one of the great choices due to its valuable source of protein and essential nutrients. In the fermentation process of *tempeh*, it can elevate the levels of isoflavones while reducing its starch and fat content. These outcomes have been linked to the reducing of blood glucose levels of T2DM, body weight and total cholesterol levels (Hsu et al., 2003; Huang et al., 2013; Lee 2006). In Kwon et al. (2010) research, the impact of fermented soybean products on T2DM was investigated. Their finding revealed that the fermentation process has not only increased the nutrient content of the raw soybean, but also broke down macromolecules into smaller peptides and amino acids and leading to enhanced sensory functions. Fermented soybean products have been linked to anti-diabetic effects attributed to the presence of various compounds including phytoestrogen, bioactive soy peptides, and isoflavonoids (Ayuningtyas et al., 2019: Murkies et al., 1998). A research on rat models by Cabello-Olmo et al. (2019) revealed that plant-based fermented foods inoculated with lactic acid bacteria (LAB) have indicated the significant impact of gut microbiota on T2DM by decreasing glucose levels and promoting more active beta cells in rat models. This findings is in accordance to Bintari et al. (2015) which have shown that both the *tempeh* and soymilk can help in lowering blood sugar level as well as increase insulin secretion. It was also observed that *tempeh* exhibited a more potent antidiabetic effect compared to soymilk due to its higher amount of biologically active isoflavones. In a similar study in 2020, insulin resistant rat model has revealed the combined impact of supplementing with *tempeh* and red ginger synergistically on insulin sensitivity in improving blood glucose levels compared to non-treated group (Dewi et al., 2020). A recent study conducted by Su et al. (2023) also revealed that consistent *tempeh* consumption as a treatment over one month exhibited significant reduction in both serum glucose levels and body weight in obese diabetic mice. Clinical studies on soy sauce, gochujang, natto (Kwon et al., 2010), kefir (Ostadrahimi et al., 2015), yogurt, (Gille et al., 2018) and kombucha (Mallmann et al., 2022) have proved to be useful for T2DM patients. However, there are none clinical evidence about diet interventions by administering *tempeh* to T2DM patients hence further studies should be addressed.

2.2. Cognitive function improvement

Soybean fermentation has been successful in producing a variety of fermented soy products that contribute to antioxidant properties, e.g., increase levels of vitamin B_2, vitamin B_{12}, and gamma-amino butyric-acid (GABA). Many studies have demonstrated the effectiveness of these vitamins, with GABA being specifically associated with the regulation of the central nervous system (Jayachandran and Xu 2019) as well as beneficial for cognitive function (Ayuningtyas et al., 2019). GABA is recognised for its role as neuro-transmitting agent which derived from the metabolic processes of glutamic acid that operates within the human brain and spinal cord (Huang et al., 2017). According to Hogervorst et al. (2008) who found that elderly Indonesians who consumed high amounts of tofu had lower memory test scores as compared to those who consumed high amounts of *tempeh* had better scores, may have been attributed to the increased levels of folate and vitamin B_{12} during *tempeh*'s fermentation process, contributing to the superior ability in improving memory. High levels of folate exhibit a protective effect on brain functionality and has been linked to a reduced risk of dementia such as Alzheimer's disease (Smith 2002).

A similar finding by Handajani et al. (2021) who conducted an intervention for six months of administering 100 gs of *tempeh* per day to elderly participants with Mild Cognitive Impairment (MCI) found advantages in enhancing cognitive functions in them. Further research by Handajani et al. (2022) has reported that the cognitive domains of memory, language, and visuospatial function of elderly were found to improve with the administration of probiotics derived from L. *fermentum*

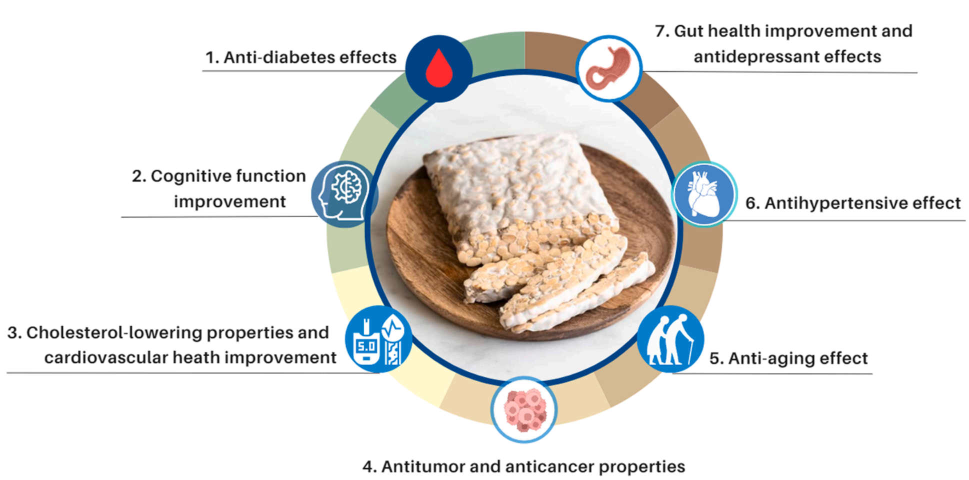

Fig. 1. Summary of health benefits on *tempeh* consumption.

isolated from *tempeh* at a concentrations of 10^8 CFU/mL and 10^7 CFU/mL, where learning process was observed to be more effectively improved at 10^8 CFU/mL. This is in accordance with the previous study by Park et al. (2020) who demonstrated that L. *fermentum* has a positive impact on the modulation of immune responses and promote health improvement as well as cognitive function. It has also been shown that fermented *tempeh* extract particularly those that have been co-fermented with *Rhizopus* and *Lactobacillus*, contained greater amounts of GABA and anthocyanins. These compounds were observed to have neuroprotective effects on a type of brain cell known as the BV-2 microglial cells which have been stimulated with lipopolysaccharides (LPS), a substance that triggers inflammation and associated with various health problems. The neuroprotective effects were achieved by decreasing the activity of nitric oxide synthase and reaction oxygen species (ROS), while also reducing the expression of the phospho-cyclic AMP response element binding protein gene, and simultaneously increasing the genes related to neurotrophic factors (Hwang et al., 2019). It has been shown in recent scientific investigations that the bioactive compounds found in *tempeh* including antioxidants and anti-inflammatory agents have been shown to have a positive influence on cognitive well-being (Handajani et al., 2022).

2.3. Cholesterol-lowering properties and cardiovascular health improvement

Soybean, as supported by several compounds such as soy protein, isoflavones and phytosterol is recognised as a healthy ingredient due to its ability to reduce serum total cholesterol and low-density lipoprotein (LDL) levels (Cho and Juillerat 2007; Ramdath et al., 2017). *Tempeh*'s protein and fiber content have been suggested to inhibit cholesterol absorption and enhance production of bile acids in human intestines (Greany et al., 2004). Numerous studies have demonstrated that consuming *tempeh* can lower cholesterol thereby promote a healthier heart. A study involving 30 individuals with hypertension and hypercholesterolemia showed that supplementation a germinated *tempeh* drink of 105 g/day, consumed in three doses/day resulted decrease in systolic blood pressure (Ansarullah et al., 2017). While in a clinical study conducted by Afifah et al., 2020, 41 women with hyperlipidemia were given *tempeh gembus* made from soymilk curd for 14 days at 103 g/day and 206 g/day showed a significant decrease in the levels of LDL at 27.9% and 30.9%, respectively and for total cholesterol at 17.7% and 19.8% while high-density lipoprotein (HDL) increased was 3.91% and 8.79%. The compounds found in *tempeh* isoflavones such as daidzein and genistein were shown to enhance the activation of LDL receptors, thereby potentially aiding in the regulation of LDL cholesterol level (Sulchan and Rukmi 2007). Recent study by Zulaikha & Kartawidjajaputra (2023) revealed that the daily intake of soy nuts and *tempeh* containing 25 *g* of soy protein for six weeks exhibited a tendency to reduce total cholesterol levels in hypercholesterolemic patients, as this protein particularly the 7S globulins have potential to modulate LDL receptors activity and inhibit hepatic Apo B synthesis (Blanco Mejia et al. 2019). Hence, daily consumption of *tempeh* may be recommended as part of healthy lifestyle for individuals with hypercholesterolemia.

2.4. Antitumor and anticancer properties

Tempeh is a soy-based fermented food that contains bioactive peptides along with abundant isoflavones such as genistein and daidzein (Tamam et al., 2019). Studies by Nurkolis et al. (2022) has shown that these compounds in *tempeh* have demonstrated potential for anticancer or antitumor effects involving the inhibition of cancer cell proliferation, suppression of angiogenesis and the induction of apoptosis in cancer cell. A few clinical and preclinical studies have demonstrated that soybeans and *tempeh* contain beneficial dietary substances that can prevent various forms of cancer. *Tempeh* isoflavones was evaluated to have anticancer activity from the test of exposing them to three types of cancer cell lines, including the breast cancer (MCF-7) (Chen et al., 2003), immortal cervical cancer (HeLa) (Xiao et al., 2011) and ovarian cancer (HO-8910) (El-Shazly et al., 2022). *Tempeh* isoflavones also significantly improved thymus index (measurement to assess the size and health of thymus gland that plays a role in the development of T cells, a type of white blood cell) and enhanced the activity of macrophages in BALB/C mice that had been implanted with S-180 sarcoma cancer cells (Mani and Ming 2017). In comparison with soybean isoflavones, *tempeh* isoflavones have been shown to have stronger antitumor activity (Mani and Ming 2017). Genistein exhibits a wide range of anticancer effect by regulating mRNA expression of several tumor suppressor genes and prevents proliferation of tumor cells (Bilir et al., 2017). Another study demonstrated the potential of genistein enhancing antitumor effect of 5-fluorouracil against pancreatic cancer making soybean fermented foods an effective therapeutic method for tumor treatment (Suzuki et al., 2014). The cancer-reducing properties of *tempeh* have also been linked to the ability of genistein in inhibiting the activity of tyrosine kinase, an enzyme that is overexpressed in many cancer cells (Varinska et al., 2015; Watanabe 1987). More clinical trials will help to access the full potential of *tempeh's* anticancer efficacy.

2.5. Anti-aging effect

Fermented soybean products have been reported to have anti-aging effects due to the presence of isoflavones genistein and daidzein (Pan et al., 2012). A study by Safrida et al. (2013) using rats as models showed that *tempeh* extract supplementation demonstrated comparable anti-aging effects to those of commercial hormone therapies such as genistein, ethinylestradiol, and somatotropin. This improvement can be attributed to the increased concentration of soy isoflavones resulting from fermentation that could replenish estrogen levels in women. Relevant studies on this reported that *tempeh* may promote anti-aging properties including enhancing skin health for both pre- and post-menopausal women (Khosravi and Razavi 2021; Sapbamrer et al., 2013). The effects are attributed to the phytoestrogens like soy isoflavones which mimicked and replenished estrogen hormones in post-menopausal women thereby enhancing the quality of their life (Das et al., 2020). Studies by Hallis et al. (2020) have confirmed the potential of *tempeh* peptide extracts as anti-photoaging cosmeceuticals through altering the expression of matrix metalloproteinase-3 gene (MMP-3) from the observed effectiveness of anti-photoaging effects in UVB-treated mice. Photoaging damages the skin by increasing activation of MMP genes which breakdown collagen directly (Bosch et al., 2015) and inducing the production of chemically reactive molecules or ions in the body known as reactive oxygen species (ROS) which if in excessive amount, can cause damage to cells, DNA and proteins (Petruk et al., 2018). Findings have revealed that the total content of L-amino acid extracted from *tempeh* protected skin from photoaging, enhanced collagen synthesis, and reduced wrinkles formation (Hallis et al., 2020; Hou et al., 2009). Further investigations are hoped to uncover the mechanisms and applications of *tempeh* peptides in the field of cosmeceuticals.

2.6. Antihypertensive effect

Muawanah et al. (2022) proposed that mung beans *tempeh* exhibits potential as a functional antihypertensive food, as fermentation leads to an increase level in Angiotensin-converting enzyme (ACE) inhibitory activity. ACE is responsible in regulating blood pressure which normally converts hormone angiotensin I into angiotensin II that has the effect of constricting blood vessel and lead to an increase in blood pressure (McCue et al., 2005). Chalid et al., al.(2019) suggested that *tempeh* protein extracts have notable anti-ACE activity, indicating their potential as functional foods for hypertension prevention and treatment. Specifically, it was found that the fraction of proteins with molecular weights less than 3 kDa contained a significant proportion of hydrophobic amino acids such as leucine, phenylalanine, alanine, and valine having the highest ACE inhibitory activity. In addition, the presence of GABA in enriched-*tempeh* makes it a promising candidate as an antihypertensive food source, as highlighted by Watanabe et al. (2006). GABA, a neurotransmitter that has been associated with potential health benefits, including antihypertensive properties which can contribute to the management of high blood pressure. It was discovered in an animal study that even a minimal intake of GABA at approximately 0.3 mg per rat per day exhibited significant antihypertensive effects in spontaneously hypertensive rats (Aoki et al., 2003).

2.7. Gut health improvement and antidepressant effects

Diet is among the most significant factor that may affect gut microbiota which can aggravate dysbiosis, a term that refers to an imbalance of microbial communities that inhabit the digestive system mostly associated with anxiety and depression (Cryan et al., 2019). Fermented foods contain prebiotics, probiotics, and biogenics components that can replenish the gut barrier by stimulating gut epithelial function and mucous production, as well as enhancing the tight junctions and barrier integrity (Maldonado Galdeano et al. 2015; Penner et al., 2005). Studies on the effects of fermented foods on gut barrier functions and mucosal immunity have largely been based on animal models (Agostini et al., 2012; Resta-Lenert and Barrett 2003). For instance, analysis of gut microbiota showed significant changes in *tempeh*-fed zebrafish, with a decrease in Proteobacteria including *E.coli* along with a tenfold-increase of Actinobacteria especially the *Bifidobacterium adolescentis* (Chen et al., 2021). This study also revealed that *tempeh* consumption led to elevated levels of brain-derived neurotrophic factor (BDNF) in zebrafish which is associated with brain health and has implications for mood regulation.

There has been strong evidence that gut microbiota directly influences brain function (Bercik et al., 2011) thus alterations in gut microbiota or direct exposure to bacteria in intestines can affect both peripheral and central nervous systems (Bienenstock et al., 2015). Probiotics from fermented food have been shown to have anxiolytic and antidepressant effects in animal studies (Dinan and Cryan 2012; Foster and McVey Neufeld 2013; Kim et al., 2016). As for immune system, Soka et al. (2015) demonstrated an increase in immunoglobulin A (IgA) in the ileum of Sprague Dawley (SD) rats after 28 days of supplementation with *tempeh,* which effect has been associated with the promote immune response due to presence of paraprobiotics (Taverniti and Guglielmetti 2011). In another study on colorectal cancer using rat model conducted by Bestari et al. (2023), it was shown that a diet rich in soy protein from *tempeh* significantly increased the population of *Lactobacillus* spp. simultaneously inhibiting the proliferation of harmful microorganisms in colon, thus give a protective effect against intestinal permeability. A further research by Stephanie et al. (2017) who employed human fecal samples and quantitative real time PCR analysis have proven that supplementation of *tempeh* have enhanced the production of IgA and increased the population of *Akkermansia muciniphila* that enhanced intestinal barrier in the human intestinal tract. Further studies are needed to evaluate the long-term effects of *tempeh* supplementation in humans.

3. *Tempeh* starter and its growth requirements

Tempeh starter, also known as *tempeh* culture, is a dried mixture of live *Rhizopus* spp. spores, a type of microfungi or mold mixed with substrate, usually soybeans. As the mold grows, it produces fluffy, white mycelia that binds the beans together to create an edible cake-like structure through partial catabolisation process of the beans. According to Nout et al. (1992), the leaves of the Indonesian Waru tree (*Hibiscus tiliaceus*) recognized locally as 'usar' were commonly used in *tempeh* production because of the presence of mycelium fungi found on the leaves of *Hibiscus tiliaceus* harboring abundance of *Rhizopus* spp.. Several studies have explored the effects of *tempeh* prepared with *Rhizopus oligosporus* (Ibrahim et al., 2002; Kameda et al., 2018; Muzdalifah et al., 2017; Watanabe et al., 2006). However there are only a few studies that have investigated on the effects of *tempeh* prepared with *Rhizopus oryzae* or *Rhizopus stolonifera* (Aoki et al., 2020; Drabo et al., 2023; Wikandari et al., 2021). Aoki et al. (2020) has compared the bioactive components in *tempeh* produced by these three different *Rhizopus* starters and demonstrated that *tempeh* fermented by *R. stolonifera* exhibited higher yields of total isoflavone aglygone and low-molecular-weight soluble dietary fiber when compared to *R. oryzae* and *R. oligosporus*. In terms of sensory characteristics, soybeans fermented with *R. oligosporus* as reported by Wikandari et al. (2021) exhibited superior qualities compared to those fermented with *R. oryzae.*

Although traditionally, fresh *tempeh* pieces can be used as starters for fermentation, this approach raises certain food safety concerns due to the potential risk of contamination and the deteriorating quality of the fungus dehydrated mycelia during storage (Wang et al., 1975). The *Rhizopus* spp. mycelium formation can be influenced by various environmental factors, including temperature, carbon dioxide (CO_2) concentration, and water activity (Sparringa et al., 2002). *Rhizopus* spp. was found to be able to thrive under low oxygen concentrations of 0.2% but unable to grow under absolute anaerobic conditions (Nout and Aidoo 2010). It was also found that certain strains were vulnerable to oxygen toxicity from H_2O_2, O_2 or OH- (Nout and Aidoo 2010). In fact, excessive aeration can lead to sporulation and drying of the beans used to make *tempeh*, which may impact mold growth negatively and result in inferior quality *tempeh* (Wilson et al., 1984). De Reu et al. (1995) revealed that mycelial growth was observed at oxygen levels as low as 0.2% (v/v) found to be inadequate for *tempeh* production. Additionally, the study found that the presence of CO_2 had a stimulatory effect at concentrations between 5 and 10% (v/v), but beyond 20% (v/v), it became inhibitory. Hocking and Miscamble (1995) discovered that three strains of *Rhizopus* spp. exhibited the most rapid growth at water activity (a_w) above 0.90. a_w is defined as the relative humidity of the gaseous atmosphere that is in equilibrium with the substrate, the significance of water activity in solid-state fermentation conditions (Oriol et al., 1988).

Prior research has shown a model that takes into account the combined impact of temperature, pH, water activity (a_w), and carbon dioxide (CO_2) concentration on the growth of mold (Sparringa et al., 2002). This study suggests that the rate of the hyphal extension which refers to the long, branching, and filamentous structure that makes up the body mycelium, was significantly influenced by pH, demonstrating a distinct peak at a pH range of 5.5 to 5.8. In comparison, the extension rate of hyphae was relatively less responsive to other factors, with the maximum rates observed at 42 °C, a_w ~1.0, and a CO_2 concentration of 0.03% (v/v). A similar finding also reported by Han & Nout (2000) that there is no discernible relationship between the impacts of a_w and gas compositions, but when considering the combination of a_w and temperature, the fungi became more sensitive to changes in temperature when there are higher levels of a_w.

4. Production of *tempeh*

Steps required to make *tempeh* are primarily a fermentation process followed by other preparatory steps. Fig. 2 shows a standard process of making *tempeh* which involves soaking soybeans in clean water, dehulling, boiling, inoculating with mold *Rhizopus* spp., packaging, and incubating. It has been found that the flow of production of *tempeh* may differ depending on the region and producer (Ahnan-Winarno et al., 2021).

Tempeh fermentation process is simple and can be easily done at home (Hachmeister and Fung 1993). Fig. 3 shows an easy guide on making *tempeh* at home by starting off with preparing fresh yellow soybeans. The soybeans must be washed to remove dust and then soaked overnight for not less than 8 hrs. During the soaking process, soybeans are hydrated and soften. This makes the dehulling process easier whilst natural acidification starts inhibiting growth of unwanted microorganisms. The dehulled soybeans with its soaking water drained away is then

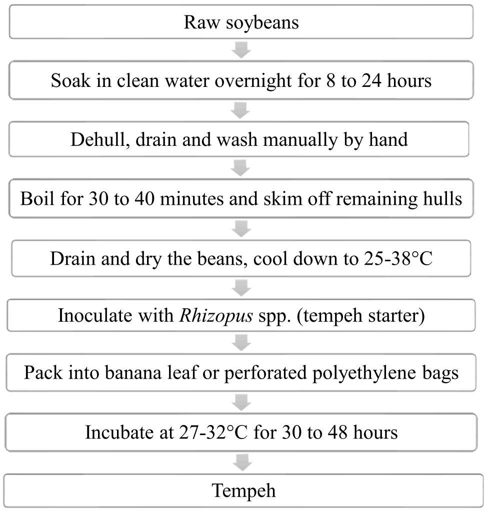

Fig. 2. The process of making *tempeh* (adapted from Ahnan-Winarno et al., 2021).

filled with fresh water again and goes for boiling for 30 to 40 min to eliminate raw taste of the soybeans and kill pathogens (Ahnan-Winarno et al., 2021). Heating the beans is also an easier way to remove the hulls. It is essential to detach the hulls from the cotyledons since intact beans cannot support mold growth (Mital and Garg 1990). After boiling, the dehulled soybeans are drained to remove excess water while it cools down to 25 to 38 °C for optimum *tempeh* fermentation. The inoculation step is by adding *Rhizopus* spp. to the soybeans cotyledons (Dinesh Babu et al. 2009; Mital and Garg 1990). The inoculated cotyledons are traditionally wrapped in wilted banana leaves to keep them moist and allow for gaseous exchange during fermentation. However, these have been replaced with perforated polyethylene bags which are more accessible in non-tropical countries and also as a convenient method for manufacturers for fermentation at 27 to 32 °C for 30 to 48 h where a dense mycelium cake will form (Ahnan-Winarno et al., 2021; Hachmeister and Fung 1993). The choice of wrapping material has an impact on the presence of volatile aromatic compounds, thereby influencing the aroma of *tempeh. Tempeh* wrapped in banana leaves consists of α-pinene that enhances beany aromas. Conversely, *tempeh* wrapped in plastic exhibited the presence of sec-butyl nitrite (cereal-like aroma), α-bisabolene (sweet and 'green' aroma) and piperazine (no significant aroma) as reported by Harahap et al. (2018). In a recent study by Erdiansyah et al. (2022), it was revealed that the composition of bacterial communities in *tempeh* can potentially influenced by various factors like manufacturing processes, raw materials and production environment but it was observed that the use of *tempeh* wraps did not result in significant changes.

Besides *Rhizopus* spp. as a starter, in recent years a few studies have found that a mixed inoculum of *R. oligosporus* with *Saccharomyces cerevisiae*, the baker's yeast in soybean fermentation indicated a significant health benefit to mankind. Rizal et al. (2021) have reported that a mixture of *R. oligosporus* and *S. cerevisiae* in fermentation resulted in higher levels of β-glucan content and exhibited antibacterial activity against *E. coli* when compared to a single culture of *R. oligosporus*. The yeast strain *S. cerevisiae* is recognised as a significant contributor of β-glucan because of its cell wall composed of β-(1,3)- and β-(1,6)-glucan, a polysaccharide that serves as a biological response modifier and regulates inflammation (Del Cornò et al. 2020) and also exhibits antimicrobial effects (Hetland et al., 2013; Rizal et al., 2021). The incorporation of *S. cerevisiae* also enhanced the aroma and concealed the undesirable earthy taste in *tempeh* (Kustyawati et al., 2018). On the other hand, soybeans inoculated with a single culture of *S. cerevisiae* without the addition of *R. oligosporus* did not show development of *tempeh* (Rizal et al., 2021). Ongoing research studies on *tempeh* in various aspects suggest substantial possibilities of developing better *tempeh* for people.

5. Substrates used for *tempeh* production

Although soybeans are the traditional and primary substrates in the production of *tempeh*, various substrates such as peanuts, chickpeas, horsebeans, wheat, and other legumes or cereals listed in Table 1 are used to produce *tempeh* (Rusmin and Ko 1974; Ashenafi 2019). *Tempeh* derived from a variety of legumes and cereals offers a highly beneficial dietary option for vegan, owing to its abundant nutritional content (Dinesh Babu et al. 2009). The elevated level of dietary fiber and unsaturated fats in legumes provide a useful source of nutrients for

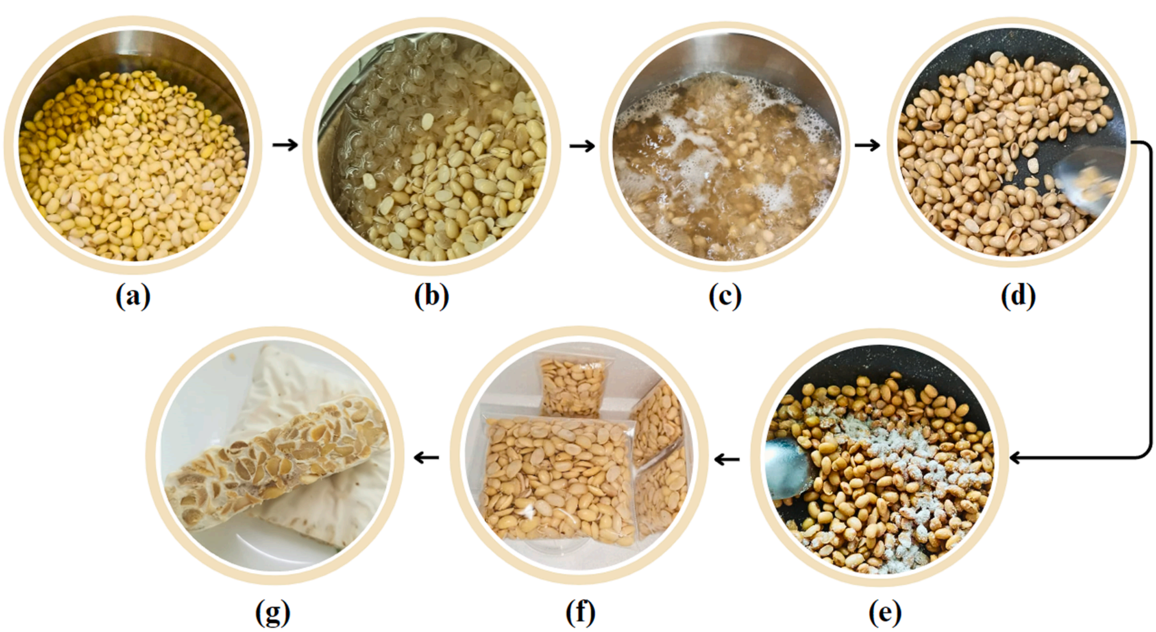

Fig. 3. A visual guide illustrating the step-by-step *tempeh*-making process, (a) Soaking, (b) Dehulling, (c) Boiling, (d) Pan-drying, (e) Inoculation with starter, (f) Fermentation and (e) *Tempeh* product.

Table 1
Substrates used for *tempeh* production using starter *Rhizopus oligosporus* mainly except for indicated ones and their nutritional effects.

Raw materials	Nutritional effects	References
(A) Legumes		
Soybeans (*Glycine* max)	Reduced antinutrient levels (trypsin inhibitor, phytic acid, stachyose and tannins) Increased vitamin B, nutritional properties, antioxidant activities and extractability of polyphenols and flavonoids	Egounlety & Aworh (2003) Santhirasegaram et al. (2016)
Mung bean or green gram (*Vigna radiata*)	Increased free amino acids, soluble phenolic acids and antioxidant activities Possessed cytotocity activities against breast cancer MCF-7 cells	Nassar et al. (2008) Norlaily Mohd et al. (2016)
Broad bean (*Vicia faba* L.)	Increased crude protein and fiber content Decreased antinutrients level	Nassar et al. (2008) Erkan et al. (2020)
[a] Chickpea (*Cicer arietinum* L.)	High values of in vitro protein digestibility Decreased phytic, acetic, fumaric and isocitric acids. Increased essential amino acids Best sensory evaluation	Reyes-Moreno et al. (2002) Abu-Salem & Abou-Arab (2011) Erkan et al. (2020)
Groundnut (*Arachis hypogaea*)	Reduced trypsin inhibitors, tannins and phytic acid	Egounlety & Aworh (2003)
Cowpea (*Vigna unguiculata*)	Reduced trypsin inhibitors, tannins and phytic acid	Djurtoft (1982); Onwuka (2006)
Cowpea + Koro bean (*Canavalia ensiformis*)	High crude protein and crude fiber content	Adhianata et al. (2022)
Kidney bean (*Phaseolus vulgaris L.*)	Reduced total phenol, phytic acid trypsin inhibitor levels	Abu-salem et al. (2014)
Black gram (*Phaseolus mungo*)	Reduced antinutrient levels and increased protein digestibility	Reddy et al. (1978) Erkan et al. (2020)
Jojoba seed (*Simmondsia chinensis L.*)	Reduced total phenol, phytic acid trypsin inhibitor levels	Abu-salem et al. (2014)
African yambean (*Sphenostylis stenocarpa L.*)	Reduced total α-galactoside	Azeke et al. (2007)
Lupine beans (*Lupinus angustifolius*)	Increased vitamin B12 and water-soluble nutrients	Nassar et al. (2008) Wolkers et al. (2018)
[b] Velvet bean (*Mucuna pruriens*)	Reduced anti-nutritional compounds and increased essential amino acids	Fitriyah et al., al. (2021)
(B) Cereals		
Wheat *(Triticum vulgare)*	Improved vitamins B, calories and protein content	Wang and Hesseltine (1966)
Barley *(Hordeum vulgare)*	Increased mineral availability and decreased phytate levels	Hachmeister & Fung (1993) Feng et al., al.(2005) Eklund-Jonsson et al. (2006)
Oat	Improved mineral availability and reduced phytate content	Eklund-Jonsson et al. (2006) Cai et al. (2014)
(C) Mixture of legumes or with nonlegumes		
Soy + Groundnut or Sunflower seed	Increased protein, free fatty acid and ash content	Vaidehi et al., al. (1985)
Black beans + Rice	High *in-vitro* protein digestibility and reduced of oligosaccharide content	Rodríguez-Bürger et al. (1998)
[c] Soy + Maize	Increased vitamins niacin, riboflavin, thiamine, dietary fiber and protein content while decreased in crude fat and carbohydrate	Mugula (1992)
Sorghum with various grains	Improved *in-vitro* protein digestibility and decreased tannin and hydrogen cyanide content	Mugula & Lyimo (2000)
Common beans + Quinoa	Increased essential amino acids and fiber content	Pilco et al. (2019)

[a] : *Rhizopus stolonifera*.
[b] : *Rhizopus* spp.
[c] : *Rhizopus oryzae* and '+' means added with.

humans, whereas cereals are also a significant source of protein, minerals and vitamins. It is observed that certain substrates such as chickpea, red lentil, and broad bean can exclusively yield high quality *tempeh* as reported by Mugula (1992) and Erkan et al. (2020).

In comparison to other legumes, the chickpea *tempeh* is considered one of the most preferred *tempeh* in terms of taste and preference (Erkan et al., 2020) and it contains high levels of protein, essential vitamins and minerals making it an excellent alternative to soy *tempeh* (Abu-Salem and Abou-Arab 2011; Reyes-Moreno et al., 2002). According to Abu-Salem & Abou-Arab (2011) and Kaur & Das (2011), the mixture of soybeans with non-soybean legumes in the *tempeh* processing tends to enhance its physical attributes such as appearance, texture, and aroma, while also positively affecting its taste, viscosity, and nutritional value.

6. *Tempeh* nutrition

Many studies have reported that *tempeh* is a highly nutritious food for humans and animals have acclaimed it to be a superfood (Romulo and Surya 2021). In general, the concentration of vitamins in *tempeh* is typically higher when compared to raw soybeans (Djurtoft and Nielsen 1983). The nutritional composition of *tempeh* varies based on the type of substrate used (Romulo and Surya 2021). For example, 100 g of fresh soybean *tempeh* contains a high amount of protein content at 20.8 g with 13.5 g carbohydrate, 8.8 g fat, 1.4 g dietary fiber and a significant amount of potassium of 234 mg (Sadewa and Murtini 2020). This numbers are consistent with the data from USDA (2023) which reported protein content of soybean *tempeh* at 20.3 g, carbohydrate at 7.64 g, fat at 10.8 g and potassium at 412 mg. The groundnut *tempeh* (*tempeh bungkil*)'s protein content is close to soybean *tempeh* at 36.27 g while other non-soybean *tempeh* such as *tempeh gembus* (solid tofu waste) and *tempeh bongkrek* (coconut dregs) have relatively lower protein and fat contents but higher in dietary fiber when compared to soybean *tempeh* (Sadewa and Murtini 2020). Besides its primarily association to the beneficial effects for the gut microbiota (Stephanie et al., 2017), *tempeh* has been demonstrated to contain vitamin B12, antimicrobial components and also antioxidant properties (Cai et al., 2014; Liem et al., 1977). This is due to various bacteria and fungus detected in *tempeh* having significant functionality as listed in Tables 2 and 3. Although mold is the primary microorganism responsible for fermenting *tempeh*, other microorganisms that present such as yeast and bacteria also proliferate during *tempeh* production (Nout and Kiers 2005). Scientific papers have highlighted the significance of bacteria found in the production of *tempeh*, for instance, *Klebsiella pneumoniae* and *Citrobacter freundii* (Keuth and Bisping 1994) have been identified as vitamin B_{12} contributor (Okada 1989). In a more recent study conducted by Kustyawati et al. (2020), it is suggested that *tempeh* fermented with starter culture consisting of *R. oligosporus* and *S. cerevisiae* exhibited more amount of vitamin B_{12} (3.15 mg per 100 g) when compared to the combination of *R. oligosporus* and *K. pneumonie* with a lower amount of vitamin B_{12} (0.81 mg per 100 g). Kustyawati et al. (2020) reported that *K. pneumonie* in *tempeh* produced enzyme β-glucosidase which hydrolysed isoflavones, daidzin into daidzen that has antioxidant properties. The significant increase of vitamin B_{12} during *tempeh* production is essential for optimal functioning of brain and formation of red blood cells (Astuti et al., 2000; Singleton and Sainsbury 2001). A recent study conducted by Priscila et al. (2020) on incorporation of *tempeh* flour as an ingredient in cereal bars, a ready-to-eat food, has led to an increased levels of protein, lipid and isoflavone aglycones content.

Table 2
Bacteria isolated from *tempeh* and its functionalities.

Bacteria	*Tempeh* Production Conditions	Functionalities	References
Klebsiella pneumoniae	Inoculated with *Rhizopus* spore at 27 °C for 1 to 2 days Inoculated with *R. oligosporus* + *C. freundii* or *K. pneumoniae* at 32 °C for 34 h Inoculated with *R. oligosporous, S. cerevisiae* and *Klebsiella sp* together and respectively at 32 ± 2 °C for 40 h	Produced vitamin B_{12} Produced vitamin B_{12} Produced enzyme to hydrolyse daidzin and genistin	Okada (1989) Keuth & Bisping (1994) Kustyawati et al. (2020)
Citrobacter freundii	Inoculated with *R. oligosporus* + *C. freundii* or *K. pneumoniae* at 32 °C for 34 h Inoculated with *R. oligosporous, S. cerevisiae* and *Klebsiella sp* together and respectively at 32 ± 2 °C for 40 h	Produced vitamin B_{12} Produced vitamin B_{12}	Keuth & Bisping (1994) Kustyawati et al. (2020)
Lactobacillus plantarum	Raw, cooked and roasted soybeans were powdered mixed with 10 ml distilled water and inoculated with L. *plantarum* for 5 days Inoculated with *R. oligosporus* spores and followed by L. *plantarum* + *Salmonella infantis/ Enterobacter aerogen/ Escherichia coli* at 30 °C for 2 days Horsebean, pea, chickpea and soybean inoculated with *R. oligosporous* spores + *L. plantarum* + *Listeria monocytogenes* respectively and incubated at 30 °C for 35–40 h Inoculate with *Rhizopus* spp. at 30 °C for 24–36 h	Reduced anti-nutritional factors by producing alpha-galactosidase Inhibited growth of pathogenic bacteria like *Salmonella infantis, Enterobacter aerogens* and *E. coli* Inhibited growth of *Listeria monocytogenes* Reduced staphylococcal enterotoxins	Adeyemo & Onilude (2013) Ashenafi & Busse (1989) Ashenafi & Busse (1992) Nout & Kiers (2005)
LactoB. brevis	Inoculated with *R. oligosporus* and *Lactobacillus* for 48 h Inoculated with *R. oligosporus* at 30 °C for 24–48 h	Reduced production of *Staphylococcal enterotoxin* Synthesis of GABA	Hachmeister & Fung (1993) Qiao et al. (2022)
Lactobacillus lactis	Inoculated with *R. oligosporus* at ambient temperature (25–32 °C)	Produced nisin and antimicrobial activity	Moreno et al. (2002)
Lactobacillus fermentum	Inoculated with *R. oligosporus* for 24–48 h Inoculated with *R. oligosporus*	Produced lactic acid Synthesised GABA	Barus et al. (2020) Handajani et al. (2022)
Lactobacillus agilis	Inoculated with *R. oligosporus* for 24–48 h	Produced lactic acid	Barus et al. (2020)
Lactobacillus delbrueckii	Inoculated with *R. oligosporus* for 24–48 h	Produced lactic acid	Barus et al. (2020)
Lactobacillus reuteri	Barley inoculated with *R. oligosporus* and LAB at 35 °C for 0–24 h	Inhibited growth of pathogenic bacteria	Feng et al. (2005)
Weissella confusa	Inoculated with *R. oligosporus* for 24–48 h	Produced lactic acid	Barus et al. (2020)
Enterococcus faecium	Inoculated with *R. oligosporus* at ambient temperature of 25–32 °C	Produced bacteriocin and inhibited growth of L. *monocytogens*	Moreno et al. (2002)

'+' means added with and '/' means or.

7. Beneficial microorganisms and its analysis in *tempeh*

Studies have shown the benefits of lactic acid bacteria and probiotics from *tempeh* for human health, there is still a lack of comprehensive research on the microbial community isolated from *tempeh*. The dominant bacteria present in *tempeh* are the Lactobacillus (LAB) families which include the *Lactobacillus plantarum, LactoBacillus brevis, Lactobacillus casei, Lactobacillus lactis, Lactobacillus fermentum, Lactobacillus agilis, Lactobacillus delbrueckii, Lactobacillus reuteri, Weissella confuse* and *Enterococcus faecium* (Radita et al., 2021). The LAB has ability to reduce pH levels from the production of acids to suppress the growth of pathogenic microorganisms during *tempeh* production (Ashenafi and Busse 1991; Moreno et al., 2002). Nurdini et al. (2015) observed the abundance of LAB during the initial stage of fermentation with low pH which had potentially hindered the growth of *Enterobacteriaceae* and bacterial spores. The presence of LAB strains and yeast did not exhibit significant inhibitory effects on the growth of *R. oligosporus* (Feng et al., 2005). It is most likely that an interaction exists among yeasts, bacteria, and molds during the fermentation process.

In researches on *tempeh* produced in the Netherlands, it was observed that the count of LAB exceeded 7 log CFU/g in 81% of the 110 total samples in the work of Samson et al. (1987) whereas Moreno et al. (2002) reported a high concentration of LAB counts in Malaysian *tempeh* ranging from 6.8 – 9.9 log CFU/g. Similarly, Djunaidi et al. (2017) found that LAB count from Indonesia *tempeh* were in the range of 8.3–8.46 log CFU/g, which is consistent with a recent study by Wikandari et al. (2021) where LAB count was observed up to 9 log CFU/g.

The applications of metagenome analysis have been useful in revealing the microbial communities in fermented food. In contrast to the research conducted in the 1980′s that primarily focused on identifying the colony forming unit (CFU) of microbes, today a diverse range of molecular biology techniques are available for studying microbial diversity. Studies on *tempeh* however are limited. Radita et al. (2017) who used 16S rRNA gene cloning technique reported that *Lactobacillus agilis, Lactobacillus fermentum* and *Enterococcus cecorum* were the predominant bacteria in *tempeh*. Pangastuti et al. (2019) used Next-Generation Sequencing (NGS) techniques and discovered microbial consortium in over-fermented *tempeh* had 17 OTUs (Operational Taxonomic Units) of fungi which refers to different clusters of fungal and 132 OTUs of bacterial communities. Microbiome study have been reviewed universally on fermented food such as sourdough (Lau et al., 2021), kombucha and kefir (Chong et al., 2023). Tenorio-Salgado et al. (2021) used a metagenomics approach to study bacterial diversity in kefir and observed a significant abundance of *Actinobacteria* while Baev et al. (2023) who studied on sourdoughs identified *Lactobacillus* as the primary genus and *Weisella* spp. was found to be the second most abundant genus of LAB in their sourdough samples.

Besides the predominant bacteria, the main microorganism in *tempeh* is still the *R. oligosporus,* the starter required in *tempeh* fermentation regardless of substrates used (Erkan et al., 2020; Mulyowidarso et al., 1989; Nout and Kiers 2005; Nurdini et al., 2015; Rusmin and Ko 1974;

Table 3
Molds isolated from *tempeh* and its functionalities.

Molds	*Tempeh* Production Conditions	Functionalities	References
Rhizopus oligosporus	Soybean inoculated with *R. oligosporus* Maize- soybean inoculated with *R. oligosporus* and *R.oryzae* at ambient temperature (28±2 °C) for 24–30 h	Produced phenolic compounds inhibited growth of pathogenic bacteria such as *Helicobacter pylori* Produced vitamin like niacin (B_3), riboflavin (B_2), pyridoxine (B_6), pantothenic acid and thiamine	McCue et al. (2003) McCue et al. (2004) Mugula (1992)
	Inoculated with *Rhizopus* spp. at 30 °C for 48 h Inoculated with *Rhizopus* spp. at 30 °C for 24–36 h	Interfered with the adhesion of *E. coli* to small intestinal brush-border membranes Exhibited antimicrobial activities against *Clostridium, Bacillus* and *Staphylococcus spp.*. Impeded growth of *Aspergillus flavus* and *A. parasiticus*	Kiers et al. (2004) Nout (1989) Nout & Kiers (2005)
Rhizopus oryzae	Soybean inoculated with *Rhizopus* spp. at room temperature for 24 h Isolated from Indonesia and Dutch *tempeh* samples	Produced amylase Reduced stachyose and raffinose, two flatulence-causing sugars by enzymatic activity	Mital & Garg (1990) Wiesel et al. (1997)
Rhizopus delemar	Isolated from Indonesia *tempeh* that inoculated with *Rhizopus* strain	Produced lactic acid and fumaric-malic acid	Hartanti et al. (2020)
Rhizopus stolonifera	Isolated from Indonesia *tempeh* that inoculated with *Rhizopus* strain	Produced organic acids and enzymes	Hartanti et al. (2020)
Mucor indicus	Inoculated with *Rhizopus* spp. at ambient temperature of 27–30 °C	Protein hydrolysis and may have contributed sweet odor and taste	Samson et al., al. (1987)

Rathbun and Shuler 1983; Samson et al., 1987). *R. oligosporus* plays a crucial role in the degradation of soybean by releasing enzymes such as carbohydrases, lipases, proteases and phytases (Barz 1995) whilst exhibit antimicrobial activities (McCue et al., 2004). Table 3 listed all molds that have been isolated from *tempeh* which include various *Rhizopus* spp. such as the *oryzae, delemar, stolonifer,* the *Aspergilus niger* and *Mucor* spp. It has been suggested that molds could have potentially contribute the flavor, texture, or nutritional properties to *tempeh* (Nout et al., 1987; Hachmeister and Fung 1993). Park et al. (2016) demonstrated that diets enriched with *R. oligosporus* has potential to enhance nutrient digestibility and intestinal environment as well as ability to produce antibiotics (Feng et al., 2005). Hamza & Gunyar (2022) shown that *R. oryzae* strains exhibited antimicrobial activities against bacterial and fungal pathogens, along with a significant tolerance to conditions within the gastrointestinal tract. These outcomes highlight that *Rhizopus* spp. are reliable probiotic sources, providing diverse benefits for health and nutrition (Hamza and Gunyar 2022).

Fermentation of *tempeh* does not only promote the growth of bacteria and molds but yeasts are also frequently found in *tempeh*. Samson et al. (1987) revealed that yeast isolated from *tempeh* in the Netherlands which include *Trichosporon beigelii, Candida lusitaniae, Candida maltose, Candida intermedia, Yarrowia lipolytica, Lodderomyces elongisporus, Rhodotorula mucilaginosa, Candida sake, Hansenula fabiani, Candida tropicalis, Candida parapsilosis, Pichia membranaefaciens, Rhodotorula rubra, Candida rugosa, Candida curvata* and *Hansenua anomola* are claimed to be non-typical for *tempeh*. The diversity of yeast during soybean *tempeh* fermentation was dependent on the fermentation method (Efriwati et al., 2013). A study conducted by Pangastuti et al. (2019) have detected various fungal species within *Trichosporon* spp. including *T. asahii, T. ovoides* and *T. gracile* were observed to involve in the degradation of proteins into amino acid compounds through hydrolysis in *tempeh* fermentation process. Mold species from the class of *Saccharomyces* such as *Tryblidiopsis sichuanensis* and *Candida* spp. were found to participate in acid fermentation during the soaking process and to provide distinctive aroma and flavor during later stages of fermentation (Pangastuti et al., 2019).

8. Reduction of antinutritional factors (ANF) in soybeans during *tempeh* processing steps

Many academic articles have been published about the production of *tempeh* and the biochemical transformations during the fermentation process. Soybean is a valuable source of dietary protein but it also contains several anti-nutritional factors (ANFs) such as phytates, tannins, trypsin inhibitors, phenols, and oligosaccharides (Adeyemo and Onilude 2013; Abu-salem et al., 2014) which can reduce the absorption of nutrients in human body (Flour et al., 2015).

Several papers have shown that pretreatment and fermentation processes can help to reduce antinutrient levels. The presence of trypsin inhibitors can hinder the proteolytic activity of the digestible enzyme trypsin, leading to a diminished supply of amino acids (Liener 1966). Onwuka (2006) has published that soaking led to a significant ($P < 0.05$) reduction in trypsin inhibitor, ranging from 28% to 30% while cooking yielded a decrease of 58% to 70% when compared with the raw beans. However, combining both the soaking and boiling/cooking process resulted in a substantial reduction of trypsin inhibitors by 95% to 98%. As shown in Table 4, it was observed that soaking for 12 h resulted in a reduction of trypsin inhibitor activity by 9.0%. Further on, dehulling of soaked soybean can lead to a significant reduction in trypsin inhibitor, and it is decreased further when cooking is performed. Cooking was found to be highly effective in deactivating trypsin inhibitor with 90% reduction. Some researchers have reported a reduction or complete elimination in the activity of trypsin inhibitors during fermentation, following an appropriate incubation period (Egounlety and Aworh 2003; Ibrahim et al., 2002; Paredes-lopez 2018).

Phytic acid is known as a major inhibitor of iron and zinc absorption. Table 4 shows that soaking beans for 12 h resulted in an increase in phytic acid content from 35.01 to 37.04 mg per g. A similar result were reported by Egounlety & Aworh in 2003, who noted that soaking beans for 12–14 h increased the level of phytic acid. This observation aligns with the research of Buckle in 1985, who found that initiating germination in whole dry soybeans led to a slight increase in phytic acid

Table 4
Effect of various treatments on soybean ANFs (trypsin, phytic acid and total phenolic) during *tempeh* production stages.

Treatment	Trypsin inhibitor	R%	Phytic acid	R%	Total phenolic	R%
Raw beans	58.13^{a} ± 0.38	–	35.01^{b} ± 0.28	–	40.60^{a} ± 0.6	–
Soaking 12 h	52.89^{b} ± 0.16	9.0	37.04^{a} ± 0.14	5.8	32.57^{b} ± 0.9	19.7
Soaking(12 h) + dehulling	50.15^{c} ± 0.38	13.7	34.33^{b} ± 0.59	2.0	29.15^{c} ± 1.6	28.1
Cooking of dehulled soaked beans	6.05^{e} ± 0.64	90.0	32.30^{c} ± 0.60	7.7	26.82^{c} ± 2.6	34.0
Tempeh	7.20^{d} ± 0.28	87.6	28.13^{d} ± 0.13	19.6	22.31^{d} ± 0.81	44.2

*Means in the same column with different letters are significantly ($p < 0.05$) different, R = Reduction Reconstructed from Abu-salem et al. (2014).

content. Moving on to the effects of dehulling after soaking, it is observed that the phytic acid content decreased by 2%, followed by a small decline of 7.7% during cooking (Table 4). Phytic acid is known to be relatively resistant to heat, thus prolonged heating is required for its destruction (Sharma and Sehgal 1992). After all the treatments for *tempeh* production, a significant decrease in phytic acid content was observed. This reduction can be attributed to the action of phytase enzyme produced by *R. oligosporus*. Such a reduction plays a vital role in enhancing the bioavailability of minerals, making *tempeh* a highly valuable nutritional source (Kurniawati et al., 2019; Riet et al., 1987).

The result in Table 4 shows that all treatments including soaking, dehulling, cooking and fermentation led to a reduction in the total phenolic content from 40.60 to 22.31 mg/g. Similar findings were observed by Kataria et al. (1989) showing a reduction in the phenolic content of mung bean after soaking. Deshpande (1982) also noted that both soaking and dehulling processes reduced the tannin content. In addition, according to Bishnoi.S et al. (1994), domestic processing and cooking methods decreased the phytic acid and polyphenol levels of different pea varieties, with a significant reduction occurring within 48 h of germination. A decrease in phenolic content indicates that microflora can ferment phenolic compounds (Elyas et al., 2002), for instance, it has been demonstrated that lactic acid bacteria (LAB) can transform phenolic compounds including flavonoids into biologically active metabolites (Filannino et al., 2015).

9. Conclusions

Tempeh, being a highly nutritious and yet cost-effective food, has not gained as much attention as it should. It has high potential a wholesome food as a plant-based protein source which is rich in essential amino acids and nutrients like fiber and vitamins. This review is aimed to provide a complete picture on *tempeh*, with supports of scientific data on it processing, health benefits and functional microbes. The primary challenge in *tempeh* processing is to create an ideal environment that fosters the growth of desired microorganisms while preventing the unwanted ones. *Tempeh* fermentation relies on the cooperative relation of bacteria and fungus, bacteria serve as the supporting cast that enhances the final *tempeh*'s nutritional quality while fungus transform soybean into cake-like structure. In the realm of microorganisms, filamentous fungus R. *oligosporus* takes the lead as the dominant player, its mycelium weaving a network that binds soybeans together, produces enzymes that break down components into more digestible forms and makes *tempeh*'s unique nutty flavor with firm texture. With a better focus, it is hoped that more researchers in *tempeh* will assist its development into a sustainable future food through optimised processing, more scientific studies on its nutrients and functional microbes and clinical studies that supports the claims on its health benefits.

Ethical statement for future foods

Hereby, I /Nyuk Ling Chin/ consciously assure that for the manuscript / A review on health benefits and processing of *tempeh* with outlines on its functional microbes/ the following is fulfilled:

1) This material is the authors' own original work, which has not been previously published elsewhere.
2) The paper is not currently being considered for publicion elsewhere.
3) The paper reflects the authors' own research and analysis in a truthful and complete manner.
4) The paper properly credits the meaningful contributions of co-authors and co-researchers.
5) The results are appropriately placed in the context of prior and existing research.
6) All sources used are properly disclosed (correct citation). Literally copying of text must be indicated as such by using quotation marks and giving proper reference.
7) All authors have been personally and actively involved in substantial work leading to the paper, and will take public responsibility for its content.

The violation of the Ethical Statement rules may result in severe consequences.

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I agree with the above statements and declare that this submission follows the policies of Future Foodsas outlined in the Guide for Authors and in the Ethical Statement.

CRediT authorship contribution statement

Sze Qi Teoh: Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Writing – original draft. **Nyuk Ling Chin:** Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing – review & editing. **Chun Wie Chong:** Resources, Supervision, Validation, Visualization, Writing – review & editing. **Adiratna Mat Ripen:** Software, Supervision, Visualization, Writing – review & editing. **Syahmeer How:** Supervision, Validation, Visualization, Writing – review & editing. **Joyce Jen Li Lim:** Data curation, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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