Review / Artigo de Revisão

Tempeh source of vegetable protein: nutritional composition and health benefits

Silvana Osorio Di Gianluca[1]
Mariel Gullian Klanian[2*]

Tempeh is a food native to Indonesia obtained from the controlled fermentation of soybeans by the fungus *Rhizopus oligosporus*. This Southeast Asian food is increasingly becoming an inexpensive substitute for meat due to its excellent nutritional profile. This review presents the most relevant aspects of the history of tempeh, the current regulation and explains the changes in soybeans' nutritional composition after the enzymatic action of the fungus. The review describes the nutritional composition and the health benefits derived from its consumption. Tempeh is a protein product registered in the CODEX Alimentarius under the CXS 313-R-2013. It contains all the essential amino acids and is an important source of B complex vitamins and calcium. During fermentation, *R. oligosporus* produces proteases, lipases, and phytases that hydrolyze various components of soybeans, releasing bioactive peptides and removing antinutritional ingredients such as phytate acid present in soybeans. Regular consumption of tempeh is associated with the prevention of several chronic diseases. Tempeh has a high level of antioxidants with a protective function against oxidative stress and decreases the signs associated with dementia and neurodegenerative disorders. Fermentation increases the content of isoflavones involved in obesity control and cancer prevention. In different countries, new strategies are being generated to position tempeh in the market as a healthy, sustainable, and accessible plant-based product.

Keywords: Tempeh, vegetal protein, nutritional composition

Running head: Tempeh: Nutritional and Health benefits

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[1]School of Health Sciences, University Marist of Mérida, Periférico Nte Tablaje Catastral 13941, CP 97300 Mérida, Yucatan México. sosorio1811014@a.marista.edu.mx

[2]Experimental Unit, University Marist of Mérida, Periférico Nte Tablaje Catastral 13941, CP 97300 Mérida, Yucatan México; +592(999)9429700.

1. INTRODUCTION

The need to obtain protein sources of high-quality vegetable origin to replace the production and consumption of meat is becoming increasingly necessary. Tempeh ("tem-pay") is an Indonesian word that collectively refers to various fermented foods based on vegetables in which the fungi of the genus *Rhizopus* play a key role in their production (Shurtleff & Aoyagi, 2020). The origin of this food dates to the Isla de Java, Indonesia, several abbreviations, including the first historical record in 1815 (Shurtleff & Aoyagi, 2007). During World War II, from 1940 to 1959, tempeh was an essential food for the Indonesian population and prisoners of war. In 1946, a Dutch prisoner of war named Roelofsen prepared tempeh inside the prison, which reduced many deaths caused by malnutrition due to a shortage of protein sources (Shurtleff & Aoyagi, 2007).

Since the 1960s, tempeh consumption has expanded worldwide and has become a preferred food for vegetarians and vegans as a meat substitute (Kiers & Nout, 2004; Permatasari et al., 2018; Aaslyng, & Højer, 2021). Many kinds of cereal, vegetables, and grains are used to prepare tempeh, with soybeans being the most common (Nout & Rombouts, 1990; Angulo-Bejarano et al., 2008; Wolkers-Rooijackers et al., 2018; Ekran et al., 2020). The preparation of tempeh with substrates other than soybeans has gained popularity since 2005, given the growing concern of diners about the consumption of that food (Ahnan-Winarno et al., 2021). The first Tempeh preparation was sourced from an accidental inoculation of *Rhizopus* spp. on the surface of the plane tree (Winarno et al., 2017).

Rhizopus oryzae was the first inoculum reported in tempeh preparation in 1895; *R. oligosporus* was identified as the best option in 1963 (Shurtleff & Aoyagi, 2020). *R. oligosporus* (*R. microsporus* var. *oligosporus*) is a filamentous fungus of the Mucoraceae family that grows in culture media containing glucose, ammonia, and salts, pH 4.0 and between 25 and 37ºC (Shambuyi et al., 1992; Nout & Kiers, 2005; Jennessen et al., 2008). It tolerates low concentrations of oxygen (0.2%) but cannot grow under anaerobic conditions. Most strains use free fatty acids as their sole source of carbon and energy; they can also use oligosaccharides such as stachyose and raffinose as carbon sources (Lin & Wang, 1991; Nout & Kiers, 2005).

During fermentation, the *R. oligosporus* is responsible for the texture and flavor of tempeh mainly through the expression of the enzymatic activity of fiber-degrading carbohydrates, proteases, and lipases. As a result of enzymatic action, soy undergoes significant biochemical modifications that improve the final product's flavor, aroma, and functional properties. With its high percentage of protein, tempeh serves as a complement to carbohydrate-based foods such as rice and replaces the primary sources of animal protein. Tempeh is not eaten raw but heated to develop the meaty flavor, it can be fried in small strips and seasoned with spices, or it can be boiled with coconut milk in soups, roasted on chopped skewers and ground into pastes with pepper (Nout & Kiers, 2005). The frying process induces the increase or appearance of the main key odors. The main aromatic compounds are 2-acetyl-1-pyrroline, 2-ethyl-3,5-dimethylpyrazine, dimethyl trisulfide, methional, 2-methylpropanal, and (E, E)-2,4- decenal (Jelen et al.,2013). Bioactive components in soy such as isoflavones and peptides play a role in the treatment of some chronic diseases. Some authors mention that Tempeh, which contains a protein composition that has been fermented into peptides (like the condition in hydrolyzed soy protein) and supported by a higher component of isoflavones than soy, has more health benefits (Nakajima et al.,2005; Haron et al., 2009).

The study aims to describe the fermentation process and the changes in the nutritional composition of soybeans after the enzymatic action of the fungus. The review provides information on the current regulation of tempeh as a food for human consumption and includes information on the health benefits derived from its consumption.

2. METODOLOGY

The information contained in this review compiles data from publications indexed in the EBSCO Publishing Science Information Database and Science Direct from 1978 to the present.

3. TEMPEH AS SOURCE OF VEGETABLE PROTEIN

3.1 Nutritional composition of tempeh

Tempeh is a protein product that contains all the essential amino acids, so its quality is like that of beef or poultry. The fermentation process breaks down the complex proteins found in soybeans, making tempeh more easily digested than unfermented soy foods or whole soybeans. It's also an excellent source of calcium.

Table 1. Nutritional composition of Tempeh products in the United States of America market (Ahnan-Winarno et al. 2021)

Nutrient	Unit	Maximum	Minimum	Description
Energy	Kcal	177.2	128.5	
Protein	g	17.7	10.9	High in protein
Total lipids	g	9.6	0.0	Low in fat
Grasa saturada	g	2.1	0.0	Low in saturated fat
Carbohydrate	g	25.3	6.4	
Sugar	g	3.7	0.0	Sugar free
Fiber	g	15.6	0.0	High in fiber
Calcium	mg	93.2	15.1	
Iron	mg	3.3	1.4	Iron source
Potassium	mg	346.1	0.0	
sodium	mg	336	0.0	Low sodium
Monounsaturated fatty acids	g	2.9	0.0	
Polyunsaturated fatty acids	g	4.0	0.0	
Trans fat	g	0.0	0.0	Free of trans fats
Cholesterol	mg	0.0	0.0	Cholesterol free
Cyanocobalamin (B12)	µg	0.1	0.0	
Thiamin (B1)	µg	0.28	0.0	
Riboflavin (B2)	µg	0.65	0.0	
Niacin	µg	2.52	0.0	

Fermentation neutralizes the phytate acid present in soybeans; therefore, tempeh does not restrict the body's absorption of minerals (Dinesh Babu et al., 2009). It is considered a product high in soluble dietary fiber and isoflavones and low in sodium. The saturated fat content of tempeh is low, and it is cholesterol-free; It also contains a high percentage of essential fatty acids and B vitamins. It is considered a product high in soluble dietary fiber and isoflavones, also low in sodium. Raw tempeh contains higher total isoflavone content than other preparations. Total isoflavone content in 100 g raw tempeh is 205 ± 56 mg dry weight and is significantly reduced to 113 ± 41 mg in 100 g fried tempeh. Raw tempeh contains 26 ± 6 mg daidzein (Da) and 28 ± 11 mg genestein (Ge), while fried tempeh contains 35 ± 11 mg Da and 31 ± 11 mg Ge/ 100 g (Haron et al., 2009).

Ahnan-Winarno et al. (2021) summarized the nutritional composition of 13 products called tempeh in the United States of America (Table 1).

3.2 Tempeh regulation as a food product

Tempeh is registered in the CODEX Alimentarius with the acronym CXS 313-R-2013 (Codex Alimentarius, 2017), which defines the product as white and compact prepared from fermentation shelled soybeans in a solid state. CODEX recognizes *R. oligosporus*, *R. oryzae*, and *R. stolonifer* as soybean fermenting fungi. The flavor of tempeh is like that of meat, mushrooms, and nuts. The smell is fresh and without traces of ammonia, and the color is white due to the growth of the mycelium of *R. oligosporus*, which gives it a compact and firm texture.

Table 2. Tempeh characteristics according to the CODEX Alimentarius Regional Standard (CXS 313-R-2013) and the Indonesian National Standard (SNI 3144:2009 2017).

Parameter	***CODEX***	***SNI***
Organoleptic characteristics		
Texture	compact	typical
Colour	white	typical
Smell	fresh, no trace of ammonia	fungus
Flavor	meat, walnut, fungus	fungus
Nutrimental composition (%)		
Moisture	< 65	< 65
Ash	-	< 1.5
Lipids	> 7	> 10
Protein	> 15	> 16
Fiber	< 2.5	< 2.5
Additives	0	-
Contamination		
External matter	no presence	-
Cadmium (mg/kg)	-	< 0.2
Lead (mg/kg)	-	< 0.25
Tin (mg/kg)	-	< 40
Mercury (mg/kg)	-	< 0.03
Arsenic (mg/kg)	-	< 0.25
Coliform (CFU/g)	-	< 10
Salmonella	no presence	no presence

(-) = without specification

The final product must not contain any food additives and must be free from contaminants like soybean hulls and other legume grains. The nutritional composition of the finished product must reach a minimum protein content of 15%, the minimum lipid content of 7%, the maximum crude fiber content of 2.5% and a maximum humidity of 65%. The pH must be in a range of 6 to 6.6 (Nout et al., 1985).

In Indonesia, tempeh has been standardized by the Indonesian National Standards Agency and registered as SNI 3144:2009 (National Standard of Indonesia [SNI], 2009). The Indonesian standard has more specifications than the CODEX Alimentarius and accepts a maximum content of contaminants such as cadmium (maximum 0.2 mg/kg), lead (maximum 0.25 mg/kg), tin (maximum 40 mg/kg), mercury (maximum 0.03 mg/kg), arsenic (maximum 0.25 mg/kg); and a maximum of 10 MPN/g of biological contaminants such as coliforms.
In its composition, the SNI includes a maximum ash content of 2.53% (p/p) and accepts a minimum protein content of 16% and lipids of 10% (SNI, 2009). Regarding moisture and fiber, both standards coincide in the percentages of fiber and moisture. Table 2 summarizes the parameters specified by both standards.

3.3 Tempeh preparation

Tempeh production has evolved over the years, and its preparation method depends on the base substrate of the fermentation. The most common procedure consists of stirring, shelling, boiling, and draining the soybeans before inoculation (Fig. 1). Soaking the grains in hot distilled water for a minimum of 6 hours provides hydration and facilitates the removal of the shell (Nout & Kiers, 2005). The grains are cooked for 30 minutes at 100ºC to eliminate microorganisms that can contaminate the product or interfere with fermentation (Nout & Kiers, 2005). Cooking also favors the elimination of antinutritional compounds present in soy, such as lecithin, tannins, and trypsin inhibitors (Nout & Rombouts, 1990; Ferreira et al., 2011). Draining and drying remove water, being the optimum humidity level 75%. Subsequently, they are left to stand until the granules reach 25ºC (Ahnan-Winarno et al., 2021).

Approximately 10^4 CFU of *R. oligosporum per* gram of substrate are used for soybean inoculation (Peñaloza et al., 1992; Nout & Kiers, 2005). Before incubation, it is necessary to provide a semi-aerobic environment using banana leaves (Owens et al., 2015) or perforated polyethylene bags to favor the growth of *R. oligosporum*. The right material allows the entry of sufficient oxygen for the development of the fungus, but not an excess of oxygen that would stimulate sporulation and darkening of the mycelium. The packaging must retain moisture from the beans during fermentation, but the tempeh must not come into direct contact with water (Steinkraus, 2018). The incubation temperature varies between 25-38ºC for 72 hours (Karyadi & Lukito, 1996; Nout & Kiers, 2005; Dinesh Babu et al., 2009).

3.4 Effect of *R. oligosporum* on soybean fermentation

During cooking, the amino acid content of soybeans is reduced by 12% due to protein denaturation (Bujang & Taib, 2014). During fermentation, *R. oligosporus* produces proteases, lipases, and phytases that hydrolyze several components of soybeans, releasing bioactive peptides and eliminating antinutritional components present in the grains (Nout & Rombouts, 1990). Enzymatic hydrolysis solubilizes soybeans' wall and intracellular material, providing optimal organoleptic characteristics to tempeh (Nout & Rombouts, 1990; Hachmeister & Fung, 1993). In addition, the degradation of macromolecules to low molecular weight substances releases active components and highly digestible free amino acids (Camacho et al., 2009; Astawan et al., 2015).

Tempeh lipases transform soybean fatty acid molecules into free fatty acids, which are substrates for the growth of *R. oligosporus.* This transformation reduces the fatty acid content of soybeans by 30% and changes the lipid profile (Ruiz-Terán & Owens, 1996). The amount of oleic acid (C18:1) and linoleic acid (C18:2) increases, and the content of linolenic acid (C18:3) decreases (Agranoff, 1999).

The main carbohydrases produced by *R. oligosporus* are polygalacturonase, endocellulase, xylanase, and arabinase (Sarrete et al., 1992). In enzymatic maceration, predominantly soybean pectin and arabinogalactan fractions are solubilized (De Reu et al., 1997), resulting in higher soybean digestibility (Wan Saidatul Syida et al., 2018). *R. oligosporus* hydrolyzes antinutritional components such as alpha-galactosides from sucrose, raffinose, and stachyose during fermentation (Nout & Kiers, 2005; Cao et al., 2007). The same occurs with polysaccharides, releasing a wide range of high molecular weight water-soluble oligosaccharides (Nout & Kiers, 2005). While many reducing substances decrease, dietary fiber increases by 3.7% (Nout & Kiers, 2005). Tempeh provides B complex vitamins due to the ability of *R. oligosporus* to biosynthesize riboflavin, niacin, nicotinamide, cobalamin, and vitamin B6 (Keuth & Bisping, 1993; Wolkers–Rooijackers et al., 2018). It also contributes to the formation of ergosterol, the precursor to vitamin D2 (Kusumah et al., 2018). Riboflavin, vitamin B6, nicotinic acid, and pantothenic acid increase during fermentation while thiamine changes very little (Murata et al., 1967). Calcium, phosphorus, magnesium, iron, copper, zinc, and manganese levels tend to increase during fermentation, mainly due to decreased levels of phytic acid, which interferes with the bioavailability of various minerals due to its chelating properties (Fung & Crozier-Dodson, 2008; Kurniawati et al., 2019).

4. TEMPEH IN DISEASE PREVENTION

Some studies have shown that the consumption of tempeh as an attractive fermented food is associated with the prevention of several chronic diseases.

4.1 Antioxidant effect of tempeh

Tempeh is rich in antioxidants with a protective function against oxidative stress, which is directly involved in the pathogenesis of several chronic degenerative diseases such as cancer, cardiovascular diseases, osteoporosis, and symptoms related to menopause (Kiers & Nout, 2004). Soybeans and their fermentation products are known to possess many isoflavones (Lin et al., 2006). Tempeh isoflavone extract has more potent free radical 2,2-diphenyl-1-picrilhidrazil (DPPH) scavenging activity than soybean isoflavone extract, while the ferrous ion chelating ability was similar for both extracts (Ahmad et al., 2015). *Lactobacillus* inoculation in the fungal fermentation process of tempeh production enhanced DPPH scavenging and antioxidant activities (Starzynska-Janiszewska et al., 2014).

In vivo studies have shown the positive effect of isoflavone consumption on antioxidant status. Balb/c mice treated for 14 days with soybean tempeh significantly improved the level of superoxide dismutase (SOD) and FRAP and reduced the level of malondialdehyde (MDA) in liver tissue (Mohd Yusof et al., 2013). Rats treated with tempeh isoflavone extract significantly elevated the level of antioxidants catalase (CAT), SOD, glutathione reductase (GRD), and glutathione (GSH) in the brain of normal and scopolamine-induced animals. The extract decreased the level of oxidative markers thiobarbituric acid reactive substances (TBARS) and nitric oxide in both models (Mani &

Ming, 2017). Another study on mice revealed that groups consuming tempeh had the more vital cognitive ability, lower levels of malondialdehyde and carbonyl protein, and higher activities of SOD and catalase (CAT) in the hippocampus, the body striatum, and cortex. Tempeh may protect neurons against oxidative stress and amyloid β-precursor protein-induced damage and reduce memory impairment by modulating nuclear erythroid factor 2 (Nrf2) through the activated protein kinase pathway by mitogen (Chan et al., 2018).

4.2 Tempeh and neurodegenerative disorders

Neurodegeneration is a series of neuronal dysfunctions due to the continuous death of neurons. The most damaging effect of neurodegeneration is memory loss. Although the evidence is scant, some studies suggest that tempeh consumption decreases signs associated with dementia. The immediate recall, is the ability to remember a small amount of information for a few seconds, is highly sensitive to dementia. A clinical survey that included older people living in rural communities in Central Java highlighted that regular consumption of tempeh foods was associated with improved immediate recall in the younger population (Hogervorst et al., 2011). Spatial learning and memory studies in rats fed tempeh isoflavone extract for 15 days showed that the time it took for the animals to consume all the baits was significantly reduced during the test. The reduced working memory error (WME) by tempeh isoflavone extract explained the improvement in short-term memory in animals. On the other hand, long-term memory improvement was represented by a significant reduction in reference memory error (RME) of tempeh isoflavone extract (Ahmad et al., 2014).

4.3 Tempeh in the treatment of obesity

Currently, mortality caused by cardiovascular diseases (CVD) is relatively high among other non-communicable diseases. Reducing elevated levels of low-density lipoprotein (LDL-C)-C is a key public health challenge. Soy protein has been extensively researched, and many studies show that its consumption lowers blood cholesterol and the concentration of total cholesterol (TC) and LDL-C in plasma (Jayagopal et al., 2002; Lee, 2006; Hsu et al., 2009; Huang et al., 2019). Fermentation increases the isoflavones and probiotics present in soy and decreases the total lipid content.

The effect of a tempeh-rich diet on cholesterol levels was reported by Mangkuwidjojo et al. (1985). Tempeh positively affected cholesterol levels and histopathological changes in the liver and arteries of rats after a 4-month feeding test. The components of tempeh inhibit the enzyme responsible for the biosynthesis of cholesterol and prevent the oxidation of low-density lipoprotein (LDL), thus minimizing plaque production in the arteries (Karyadi & Lukito, 1996; Astawan et al., 2015).

The use of tempeh as a dietary supplement is potentially beneficial for abnormal metabolism. The triglyceride levels markedly reduced in patients with diabetes type II consuming 2 g of tempeh capsules daily over three months (Su et al., 2021). Consumption of tempeh enriched with γ-amino butyric acid (GABA) decreased the level of triacylglycerols in blood plasma compared to soy protein and casein. Increased HDL-C and decreased LDL-C levels in the GABA-enriched tempeh group favored the anti-atherosclerosis effects of GABA-enriched tempeh (Watanabe et al., 2006). Reduced serum cholesterol was also demonstrated in hypercholesterolemic rabbits fed an alcoholic extract of tempeh (Hermosilla et al., 1993).

4.4 Tempeh on malnutrition

Some authors studied tempeh as the main source of nutrients in the treatment of malnutrition. Fermented soybean cake nugget was used as an alternative for increasing weight of little children aged 36–60 months (Suriani et al., 2021). In a randomized controlled clinical trial conducted in Indonesia with malnourished patients aged 1 to 10 years, supplementation with a tempeh-based food formula increased serum iron concentration and hemoglobin (Iva et al., 2012). This formula obtained results like those of the F100 milk formula created by the World Health Organization (Iva et al., 2012), the gold standard for treating severe malnutrition in infants under six months (World Health Organization, 2005).

4.5 Antidiarrheal and immunomodulatory effects

Tempeh has immunomodulatory and immunostimulant properties. IgA gene expressions and IgA protein secretion in rats supplemented with raw and cooked tempeh are significantly higher than in the control group (Soka et al., 2015). The decrease in oligosaccharide content, especially raffinose and stachyose during fermentation, eliminates flatulence problems caused by soy consumption. Furthermore, given its high content of isoflavones, tempeh has an immunomodulatory effect, improves intestinal immunity, and decreases the duration of diarrhea (Karyadi & Lukito, 1996). A landmark report by van Veer & Schaffer (1950) found that Java Island prisoners consuming tempeh remained free of gastrointestinal infections despite poor blood pressure hygiene. Later studies by Partawihardja (1990) in infants aged 6 to 24 months found that infant formulas made with tempeh reduced the duration of diarrhea, resulting in increased body weight and overall improvement in nutritional status. During the following three months, an observation period consuming the same food formula favored the average linear growth in children with malnutrition (Suigbia et al., 1990).

4.6 Tempeh and Cancer Prevention

Numerous preclinical and clinical trials have reported the effect of beneficial dietary substances in soybeans and tempeh on various types of cancer. High consumption of soy products may modulate carcinogenesis, that is, the initiation, promotion, and progression of hormone-dependent cancers, including breast and prostate cancers.

Kiriakidis et al. (2005) isolated several isoflavones from tempeh extract, identifying them as genistein, daidzein, 6,7,40-trihydroxyisoflavone, 7,8,40-trihydroxyisoflavone (7,8,40-TriOH), and 5,7,30, 40-tetrahydroxyisoflavone (orobol). The effect of isoflavones was analyzed in reducing angiogenesis using a chicken chorioallantoic membrane. All isolated isoflavones inhibited angiogenesis, mainly genistein, which reduced it by 75.09%, followed by orobol (67.96%).

Xu et al. (2002) isolated genistein, daidzein, glycitein, genistein, and daidzin from tempeh isoflavones. They exposed them to three cancer cell lines, including MCF-7 (breast cancer cell line), HeLa (immortal cervical cancer cell), and HO-8910 (ovarian cell). Genistein significantly inhibited tumor cell lines among the isolated compounds, and daidzein affected the HO-8910 cell line. In addition, tempeh isoflavones inhibited tumor growth with an inhibition rate of 30.9% and significantly improved thymic index and macrophage activity in BALB/C mice implanted with S-180 sarcoma cancer cells. In general, tempeh isoflavones had more potent antitumor activity than soy isoflavones.

5. CONCLUSION

Currently, tempeh is the subject of new studies as a high-protein, low-cost food. In different countries, new strategies are being generated to position tempeh in the market as a healthy, sustainable, and accessible plant-based product (Aaslyng, & Højer, 2021). Some of these initiatives aim to position tempeh internationally; an example of this is the Tempeh Movement, which focused on the ecological aspect, highlighting that tempeh is a sustainable and environmentally friendly food (Ahnan-Winarno, 2019). This review showed that tempeh is a food that may be relevant for food security, mainly for its nutritional composition, low cost, and benefits for human health.

Tempeh fonte de proteína vegetal: composição nutricional e benefícios para a saúde

Resumo

Tempeh é um alimento nativo da Indonésia obtido a partir da fermentação controlada da soja pelo fungo *Rhizopus oligosporus*. Este alimento do Sudeste Asiático está se tornando cada vez mais um substituto barato para a carne devido ao seu excelente perfil nutricional. Esta revisão apresenta os aspectos mais relevantes da história do tempeh, a regulamentação atual e explica as mudanças na composição nutricional da soja após a ação enzimática do fungo. A revisão descreve a composição nutricional e os benefícios para a saúde derivados de seu consumo. Tempeh é um produto proteico registrado no CODEX Alimentarius sob o CXS 313-R-2013. Contém todos os aminoácidos essenciais e é uma importante fonte de vitaminas do complexo B e cálcio. Durante a fermentação, R. oligosporus produz proteases, lipases e fitases que hidrolisam vários componentes da soja, liberando peptídeos bioativos e removendo ingredientes antinutricionais como o ácido fitato presente na soja. O consumo regular de tempeh está associado à prevenção de várias doenças crônicas. Tempeh tem um alto nível de antioxidantes com função protetora contra o estresse oxidativo e diminui os sinais associados à demência e distúrbios neurodegenerativos. A fermentação aumenta o conteúdo de isoflavonas envolvidas no controle da obesidade e na prevenção do câncer. Em diferentes países, novas estratégias estão sendo geradas para posicionar o tempeh no mercado como um produto à base de plantas saudável, sustentável e acessível.

Palavras-chave: Tempeh, proteína vegetal, composição nutricional

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