International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 14, Issue 6, 2022

Original Article

FERMENTATION OF SOYBEAN SEEDS USING *RHIZOPUS OLIGOSPORUS* FOR TEMPEH PRODUCTION AND STANDARDIZATION BASED ON ISOFLAVONES CONTENT

YOHANES DWIATMAKA[1,2*], NUNUNG YUNIARTI[1], ENDANG LUKITANINGSIH[1], SUBAGUS WAHYUONO[1]

[1]Doctoral Program, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia, [2]Faculty of Pharmacy, Sanata Dharma University, Yogyakarta, Indonesia
*Email: atma14@usd.ac.id

Received: 07 Dec 2021, Revised and Accepted: 14 Aug 2022

ABSTRACT

Objective: To determine the best temperature and time of fermentation for making soybean (*Glycine max* (L.) Merril) tempeh seeds with high content of isoflavones.

Methods: Five varieties of soybean seeds, Devon-1, Dena-1, Dega-1, Anjasmoro, and Argomulyo, were determined for their isoflavones content using an Ultraviolet (UV) spectrophotometer. A variety containing the highest isoflavones was washed, boiled, peeled, then mixed with tempeh starter (*Rhizopus oligosporus* culture) at 1 g/kg. The mixture was then poured into plastic bags and flattened with two centimeters of thickness. Fermentation in three conditions: (a) ambient temperature (27-32 °C) without air circulation, (b) 27±0.5 °C, and (c) 30±0.5 °C both with air circulation. The inner temperature, ripening time, and rotting time was recorded. The total isoflavones content was measured every 6 h.

Results: The variety of Devon-1 has the highest content of isoflavones (0.112% w/w). Fermentation in condition (a) caused the tempeh too hot (42 °C) and rotted at the 42nd h. Condition (b) produced the best tempeh, ripening at the inner peak temperature (32.5 °C) at the 32nd h; and rotted after the 100th h. Condition (c) produced good tempeh; the ripening occurred at the 31st h at 33 °C and rotted after the 113th h. Tempeh that was produced with condition (b) at the 72nd h has the highest content of isoflavones (0.089% w/w).

Conclusion: Fermentation at 27±0.5 °C with air circulation for 72 h produced tempeh with the highest isoflavones content (0.089% w/w), but decrease about 20% compared to its content in seeds (0.112% w/w).

Keywords: Tempeh, Soybean, Fermentation, *Rhizopus oligosporus*, Isoflavones

DOI: https://dx.doi.org/10.22159/ijap.2022v14i6.43785. Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Tempeh is an Indonesian traditional food, commonly made from white soybeans by fermentation with *Rhizopus* molds. Soybean tempeh is a very nutritious food and very familiar to Indonesian people. Soybeans as the raw material of tempeh have very good nutritional components [1]. Soybean is often called "golden from the soil" because of its high, complete, and balanced protein content [2]. Soybean is also known as golden beans [3]. Soybean also contains lunasin, a polypeptide that can inhibit the expression of COX-2 enzyme in cryptic epithelial cells of the distal colon in mice that have been induced with carcinogen [4]. Soybean extract combined with probiotics significantly improves women's climacteric symptoms and sexuality [5]. The quality of spermatogenesis in newborn Wistar mice will reduce when their mothers are administered soybean tempeh ethanol extract from preovulation until the weaning season [6]. In addition to nutrition content, soybeans also contain high secondary metabolites of isoflavones. Isoflavones can act as antioxidants and phytoestrogen that improve memory [3].

An environment with more pollution will increase the number of free radicals in the body, thus accelerating deterioration, tissue damage, and cell death [7]. Free radicals trigger aging and various degenerative diseases. The antioxidant activity of soy isoflavones has good potency to reduce the damage of body cells. Consuming soybean products can increase the concentration of the superoxide dismutase (SOD) enzyme and the total antioxidant status [8].

Estrogens are distributed in the body, including the central nerve. Estrogens affect cognitive abilities because they modulate the central nervous system's neurotransmitters, including acetylcholine, catecholamine, serotonin, and gamma-aminobutyric acid (GABA). Estrogen receptors are present in various parts that are involved in cognitive processes, such as the formation of the hippocampus, amygdala, and cerebral cortex, as well as learning and memory processes [9]. Decreasing estrogen concentration in the body, such as during menopause, causes memory loss. Estrogen replacement therapy can improve episodic and semantic memory skills. Estrogens will induce synaptogenesis in the hippocampus. Soybean seeds contain quite a lot of isoflavones, especially genistein, daidzein, and glycitein that can act as a phytoestrogen. These isoflavones and some of their metabolites have agonist activity or partial agonists against estrogen receptors so they can act as weak non-steroidal estrogens [8,10]. Phytoestrogens, including genistein and daidzein, have estrogenic activity with a higher affinity for β-estrogen receptors caused by their structural similarity to 17β-estradiol. Genistein and daidzein were able to suppress bone loss too [11]. In non-equol producer women, supplementation of daidzein did not significantly decrease the menopausal symptoms nor improve their quality of life [12]. Isoflavones are also able to improve cognitive function through the protective mechanism of the nervous system [13]. These make it interesting to study the ability of soy products to improve memory.

In soybean seeds, there are at least 12 types of isoflavones related to genistein, daidzein, and glycitein [14, 15]. The total content of isoflavones in dry soybeans reaches 0.1-0.4% [14]. In other condition it can even reach between 0.4-9.5 mg/g or 0.04-0.95% [16]. Genistein is more dominant than other isoflavones [17]. The processing of soybeans may reduce the content of isoflavones, but commonly people consume in the form of processed food products such as tempeh. Fermentation of soybean seeds during tempeh production reduces non-nutritional substances and increases the content of vitamins. Fermented soybeans also serve good protein and calcium, low in saturated fat, folic acid, and sodium [18]. In another case, fermentation of soybeans with *Bifidobacterium animalis* able to increase the content of isoflavone aglycones, amino acids, peptides, protein, and sugar compared to fresh soybean [19]. Fermented soybeans have good potency of nutritious food [18]. Fermentation also improves the quality of active phytochemicals and makes the human digestive system easier to absorb them [19]. Fermentation with *Rhizopus* molds during the process of tempeh production also hydrolyzes the isoflavone glycosides to be free of isoflavones (isoflavone aglycones) [20]. Isoflavone aglycones are more easily absorbed in the human digestive system and provide a quick effect [21, 22]. The process should be controlled to get a good quality of tempeh. Some parameters need to be considered, including the fermentation temperature and fermentation time [25]. The specific content of genistein and daidzein in soybean can be analyzed using reverse-phase high-performance liquid chromatography (RP-HPLC) coupled to PDA at 254 nm [23]. There is a simpler method to determine isoflavones content using ultraviolet (UV) spectrophotometry [24].

It is very important to make tempeh with good quality and standardized isoflavones content. Fermentation during tempeh production needs several suitable conditions. This research was aimed to determine the best room temperature and time of fermentation to get tempeh with high content of isoflavones.

MATERIALS AND METHODS

Materials

Certified soybean seeds were obtained from the Indonesian Legumes and Tuber Crops Research Institute ('*BALITKABI: Balai Penelitian Tanaman Aneka Kacang dan Umbi*'), Malang, East Java, Indonesia. Soybean seeds come in 5 varieties: Devon-1, Dena-1, Dega-1, Anjasmoro, and Argomulyo. Genistein (Sigma-Aldrich, USA), ethanol (Merck and Co., Inc., Germany), distilled water.

Making soybean tempeh

Five varieties of soybean seeds were determined for their isoflavones content. The highest content of total isoflavones was then processed as tempeh. The tempeh was made through several stages [26] with a reduction of soaking time and the second boiling time. The process was started by sorting and washing the soybean seeds. The next step was first boiling for 30 min, then ground to break the seeds and separate the husks. The seeds were washed and soaked in fresh water for 12 h. The next steps were second boiling for 30 min, then draining the water and letting the seeds become warm. Soybean seeds were then mixed with tempeh starter (mold of *Rhizopus oligosporus* culture) at 1 g/kg, then pour into plastic bags and flattened with two centimeters of thickness. The sterile needle was used to perforate the plastic bags one centimeter apart. The last step was to incubate the mixture in the incubator and let the fermentation occur under necessary conditions. Three conditions were applied: (a) fermented at ambient temperature (27-32 °C) without air circulation, (b) 27±0.5 °C, and (c) 30±0.5 °C both with air circulation. The inner temperature of tempeh was measured continuously using Elitech® RC-4 mini temperature data logger. The total isoflavones content of tempeh was measured every six hours.

Analysis of isoflavones content

A total of 30.0 g of fresh tempeh powder and soybean seeds powder were extracted with absolute ethanol to obtain 50.0 ml extract. Then 100 µl of tempeh extract was diluted in ethanol to 10.0 ml. For soybean powder, 50 µl of the extract was diluted in ethanol to 10.0 ml. Then 3.0 ml of each solution was poured into the cuvette to determine the isoflavones content. Total isoflavones were determined using a UV spectrophotometer (SHIMADZU UV-1800) at 262 nm.

Ultraviolet absorption spectral scan

For both tempeh and soybean extracts, scanning was carried out in the range of 200 to 400 nm using a Spectrophotometer (SHIMADZU UV-1800). Scanning was performed to obtain the spectral pattern of each material, compared to the genistein spectral pattern as standard.

Statistical analysis

All the parameters were expressed as mean±SD and one-way ANOVA was carried out followed by Tukey or Games-Howell test. The difference between the groups was considered significant at $p<0.05$. Analysis using statistical software Jamovi version 1.6.23 (https://www.jamovi.org).

RESULTS

Isoflavones content in five varieties of soybean seeds

There are 5 varieties of soybean seeds selected: Devon 1, Dena 1, Dega 1, Anjasmoro, and Argomulyo. They have different total isoflavones content.

Table 1: Total isoflavones content in 5 varieties of soybean seeds

Soybean varieties	Isoflavone content* (% w/w)	Rank
Devon 1	0.112±0.0012^{a}	1**
Dena 1	0.062±0.0009^{e}	5
Dega 1	0.092±0.0011^{b}	2
Anjasmoro	0.068±0.0009^{d}	4
Argomulyo	0.074±0.0015^{c}	3

*Means from three determinations±standard deviation followed by the same uppercase letter in the same column do not differ by the Tukey test (P<0.05); **Selected for tempeh production.

As shown in table 1, the variety of Devon-1 has the highest isoflavones content, followed by Dega-1, Argomulyo, Anjasmoro, and Dena-1. The isoflavones content was significantly different.

Fermentation process

Without good air circulation, fermentation at an ambient temperature of 27-32 °C (condition a) caused tempeh too hot even up to 42 °C and rotted rapidly (fig. 1). At the 42nd h of fermentation, the tempeh showed signs of decay, marked by the smell of ammonia, brown spots, and softer texture, then the fermentation was stopped.

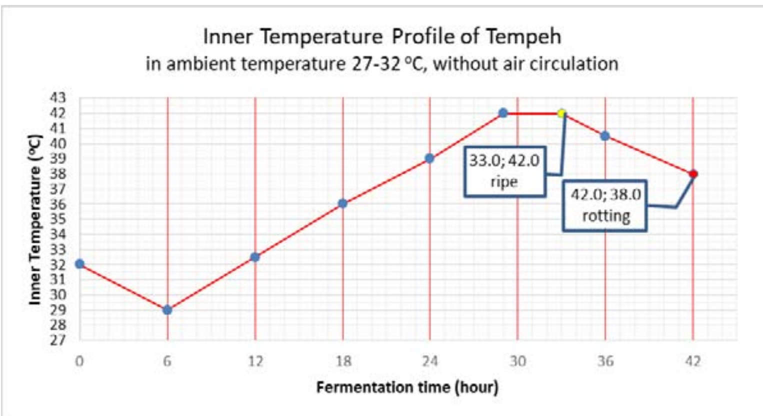

Fig. 1: Inner temperature profile of tempeh during fermentation period, in ambient temperature (27-32 °C) without air circulation (condition a)

When fermented at 27±0.5 °C with air circulation (condition b), the inner peak temperature was 32.5 °C reached at the 32nd hour as well as the time for tempeh to ripe (fig. 2). With controlled temperature and air circulation, the inner temperature profile of tempeh has a parabolic-like pattern, especially at the time range for tempeh ripening. Rotting started at the 100th hour with an inner temperature of 31.9 °C.

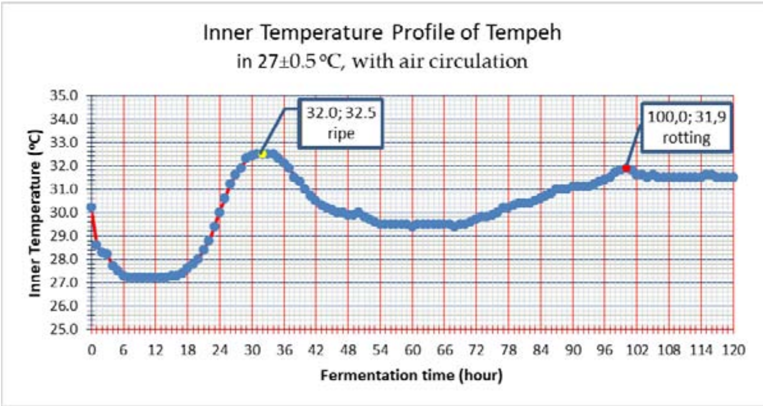

Fig. 2: Inner temperature profile of tempeh during fermentation period, at 27±0.5 °C with air circulation (condition b), The fermentation was also carried out at 30±0.5 °C with air circulation (fig. 3)

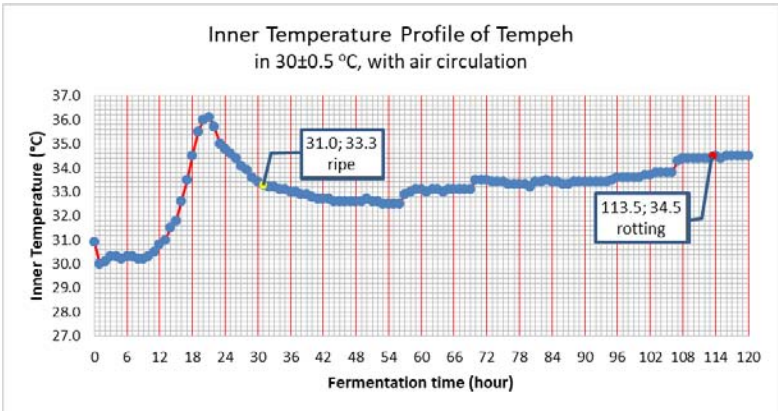

Fig. 3: Inner temperature profile of tempeh during fermentation period, in 30±0.5 °C with air circulation (condition c)

While fermented at 30±0.5 °C (condition c), the inner peak temperature of tempeh was 36 °C (at the 22nd h), but the ripening occurred after the temperature dropped to 33 °C at the 31st h. Rotting started at the 113th h with an inner temperature of 34.5 °C (fig. 3). Tempeh is ripe when *Rhizopus* mycelium covers the entire surface of tempeh. At that time, tempeh would be white, clean, and good smell. When the tempeh starts to rot, the color is darker, some brown areas appear and ammonia odors smelt (fig. 4).

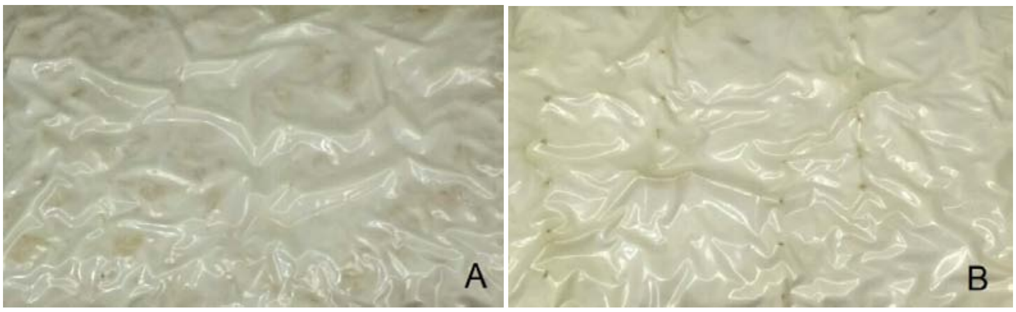

Fig. 4: Tempeh condition: ripe (A) and start to rot (B)

UV absorption spectra

The ethanol extracts were scanned at UV wavelength (200-400 nm). There was a similar spectral pattern among genistein, soybean extracts, and tempeh extracts (fig. 5). They have UV spectral characteristics of isoflavones.

Isoflavones content in tempeh

The isoflavones content in tempeh changes along the fermentation time. The highest content of isoflavones was during the fermentation time at 96 h but has no significant difference from the 72 h (table 2).

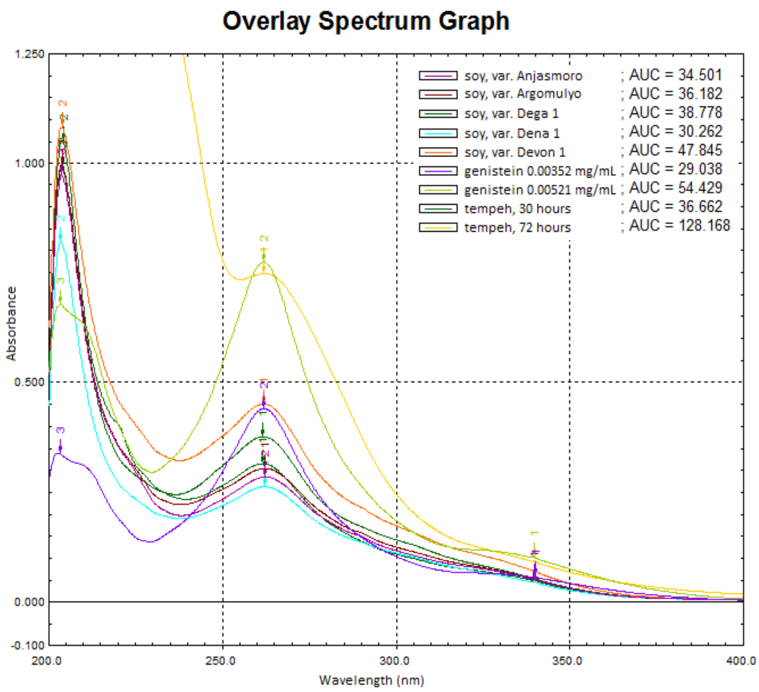

Fig. 5: Overlay Uv-spectra of soybean extract, tempeh extract, and genistein. (Area under the curve (AUC) were counted manually from the spectrum, using the trapezoid method)

Table 2: Isoflavones content in tempeh with various fermentation times at condition b (27±0.5 ℃)

Fermentation time (h)	Isoflavones content* (% w/w)	Rank
0	0.034±0.0009^{a}	15
6	0.035±0.0004^{a}	14
12	0.032±0.0003^{a}	17
18	0.033±0.0005^{a}	16
24	0.043±0.0002^{ab}	12
30	0.037±0.0003^{a}	13
36	0.067±0.0242^{de}	9
42	0.052±0.0004^{bc}	11
48	0.057±0.0043^{cd}	10
54	0.070±0.0027^{e}	7
60	0.077±0.0019^{e}	6
66	0.078±0.0014^{ef}	4
72	0.089±0.0128^{fg}	2**
78	0.070±0.0013^{e}	8
84	0.078±0.0012^{e}	5
90	0.079±0.0013^{f}	3
96	0.093±0.0051^{g}	1

*Means from three determinations±standard deviation followed by the same uppercase letter in the same column do not differ by the Games-Howell test (P<0.05); **Selected fermentation time.

DISCUSSION

Five varieties of soybean seeds were selected, namely: Devon-1, Dena-1, Dega-1, Anjasmoro, and Argomulyo. Farmers commonly cultivate those varieties and use them as raw materials for making tempeh and tofu. Those soybean seeds were very good quality and certified by BALITKABI, Malang, Indonesia. Soy phytonutrients that are reported to mainly contribute to memory ability are isoflavones, especially genistein and daidzein, through their role as phytoestrogen [10] and antioxidants [27, 28]. Tempeh of good quality was prepared by using good soybean seeds. Therefore, the first step was to determine the isoflavones content in soybean seeds of the 5 varieties. This research showed that the variety of Devon-1 has the highest content of isoflavones (table 1). It was consistent with the earlier researchers [14, 16]. Soybean seeds of Devon-1 then proceed to make tempeh with high content of isoflavones. Soybean seeds that contain high isoflavones it is expected to produce tempeh with high isoflavones content as well.

At the step of first boiling for 30 min, soybean seeds would be softer and cause easier to peel the seed coat. Boiling, of course, results in the solubility of isoflavone glycosides in the cooking water. This result was in line with a previous study [29]. Grinding to break the seeds and separate the seeds coat was the next step. At that step, most of the seeds would split into two pieces, even in the form of quite small pieces. This would further enhance the contact surface area between seed pulp and water during the soaking step. The next step was soaking the soybean seeds for 12 h so the seeds become softer. According to previous research [30], natural acidification occurred during the soaking step so it will reduce the pH value. This step causes some of the isoflavone glycosides to dissolve in the soaking water. It was physically observed that the immersion water become yellowish [31] due to the dissolution of some isoflavone glycosides. Although, according to earlier research [32] soaking was not reducing the carbohydrate and polyphenol but decreased the protein and increase the lipid content. The second boiling was performed for 30 min as well. In this step, it was certain that some of the isoflavone glycosides dissolve again in the cooking water. This of course, reduces the isoflavones content in the seeds again. The last step of tempeh production was fermentation after mixing soybean seeds with tempeh starter (culture of *Rhizopus oligosporus* mold). Finally, the total isoflavones level of Devon-1 seeds (table 1) would decrease about 20% when it has become tempeh within 72 h of fermentation (table 2). During the fermentation process, the β-glycosidase enzyme from *R. oligosporus* hydrolyzes isoflavone glycosides to be isoflavone aglycones. This hydrolysis process has been described by several researchers [30, 33, 34]. Rhizopus also produces minor phenolic and release fiber-bound phenolic substances [30]. Isoflavone glycosides also can be hydrolyzed in the digestive tract [21], but it certainly takes a while and may not be perfectly hydrolyzed. In the form of tempeh, most of the isoflavone glycosides have been hydrolyzed into isoflavone aglycones [34]. Fermentation as the last step of tempeh production is very important because isoflavone glycosides are hydrolyzed to be isoflavone aglycones.

Adult people are not able to absorb isoflavone glycosides, but the aglycons are absorbed [35], so by consuming tempeh, the benefits of isoflavones will be better obtained. Therefore, the loss of isoflavone glycosides during the process of making tempeh was compensated by the formation of isoflavone aglycones that are more active in the human body. In tempeh, most macromolecules form also have been hydrolyzed to be simpler, thus increasing the nutritional value of tempeh compared to soybeans without fermentation [36]. *Rhizopus* molds during tempeh fermentation, produce enzymes that can hydrolyze protein, lipid, and starch. Hydrolysis of protein increases the content of amino acids and low-molecular-weight peptides [37]. Hydrolysis of macromolecules causes the degradation of the seed cell's structure that making the tempeh softer and more desirable as a food product.

Below 27 °C the fermentation process will be slow because low temperature inhibits the growth of *Rhizopus* molds [38]. This condition would be harmful because it allows contaminant bacteria to grow faster than *Rhizopus*. In this study, when the fermentation temperature was maintained at 27±0.5 °C with air circulation, the fermentation process runs normally and the tempeh would be more durable. In the other study [39], fermentation can also be performed at a higher temperature, 30 °C, also at 31 °C with a relative humidity of 92% [40] and both were no air circulation explained. Here in this research, fermentation at ambient temperature (27-32 °C) and without air circulation caused the tempeh too hot and rotted quickly (fig. 1). Exactly, *R. oligosporus* produces β-glucan as antibacterial [41], but when the inner temperature is too hot, the growth of *Rhizopus* is inhibited and mesophilic or thermophilic bacteria will grow quickly causing tempeh to rot. Nevertheless, at 30±0.5 °C with air circulation (fig. 3), it produced good tempeh. Air circulation was able to distribute heat and moisture on the surface of tempeh. This research shows that air circulation on the surface of tempeh during fermentation has an important role in lowering the inner temperature of tempeh. Ventilation has the same effect [25]. Increasing fermentation temperature caused the inner peak temperature to reach earlier. Although the fermentations were carried out at different temperatures, the ripening time was relatively stable, occurring from the 31st to the 33rd hour (fig. 1, 2, and 3). It seems that ripening time was not temperature-dependent. In those conditions, the inner temperature of ripe tempeh was 32-33 °C. Without air circulation and fermented at ambient temperature (the rate was 28 °C), tempeh would ripe at 42 °C, although the ripening time remains at 33 h, the tempeh rotted quickly. Producing good soybean tempeh needs optimum room temperature to minimize energy consumption. This study showed that the room temperature between 27±0.5 °C to 30±0.5 °C with air circulation would be a suitable condition to produce good tempeh. Fermentation at 27±0.5 °C (condition b) was more suitable for tempeh production than fermentation at 30±0.5 °C (condition c). Fermentation at condition b, ripening occurred at the inner peak temperature (fig. 2). It indicated that fermentation runs smoothly and *Rhizopus* molds grew better than fermented at condition c. By condition c, the inner peak temperature occurred before the tempeh ripened and was higher than its ripening temperature. This condition was not good for *Rhizopus* growth. It can increase the risk of overheating and cause the failure of the fermentation. Based on this research, fermentation at 27±0.5 °C with air circulation was chosen to produce tempeh with the standardization of isoflavones content. Therefore, the isoflavone content is only presented from tempeh fermented in condition b (table 2).

Ultraviolet absorption scanning results showed that the UV spectra pattern of soybean extracts and tempeh extracts have similarities with a spectrum of genistein standard solution. These spectra correspond to the spectrum of soybean isoflavones especially genistein and daidzein [16]. This suggested that the main component in soybean extracts and tempeh extracts was isoflavones. There were different quantities of isoflavones, indicated by their differences in optical density and area under the curve values ($AUC_{200\text{-}400\ nm}$) (fig. 5). The UV spectrum of the extract can be used as a simple identification for soybean products. Good soybean products should have the characteristic of the isoflavones UV spectrum.

Soybean seeds have good potency as a material of functional food since the high content of isoflavones and capacity of antioxidants [1]. Soybean tempeh contains complete nutrients [2] and isoflavones that can improve memory [3]. For these reasons, tempeh has a good chance to be processed as a functional food to improve memory. As a functional food, tempeh should have a standard of isoflavones content. Tempeh with high content of isoflavones has to be designed from the starting process by choosing the best soybean variety. Based on this research, a variety of Devon-1 was chosen to produce tempeh, because its total isoflavones content was higher than the four others (table 1). Fermentation at 27±0.5 °C with air circulation produced tempeh with the highest content of isoflavone at the 72nd hour of fermentation time, with no significant difference to the 96th hour (table 2). Tempeh at the 72nd hour of fermentation was a very good condition, suitable for consumption, and of course, has a shorter time process. For these reasons, tempeh at 72nd-hour fermentation was chosen as a tempeh product that standardized the total isoflavones content. It is interesting to investigate further because the changes in total isoflavones levels in tempeh are not linear with the increasing fermentation time (table 2).

CONCLUSION

The process of making tempeh will reduce the total isoflavones content by about 20%. The good fermentation temperature for making soybean tempeh is around 27-30 °C and with air circulation. The ripening of tempeh is reached at the fermentation time between 31-32 h, with the inner temperature around 32-33 °C. The best condition of tempeh with the highest isoflavones content is fermentation for 72 h at 27±0.5 °C.

ACKNOWLEDGMENT

The authors are grateful to the Kementerian Riset Teknologi Dan Pendidikan Tinggi Republik Indonesia by Lembaga Pengelola Dana Pendidikan (PRJ-4772/lPDP.3/2016) as funder of this research; Indonesian Legumes and Tuber Crops Research Institute ('BALITKABI: Balai Penelitian Tanaman Aneka Kacang dan Umbi'), Malang, East Java, Indonesia for supplying the soybean seeds.

AUTHORS CONTRIBUTIONS

YD performed research activities and prepared the manuscript. EL involve in the methodology and validation. NY writing review and editing. SW writing-review, and supervision. All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTERESTS

The authors declare no conflict of interest. The funders had no role in the design of the study; in the data collection, analysis, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

REFERENCES

1. Seo WD, Kang JE, Choi SW, Lee KS, Lee MJ, Do Park KD. Comparison of nutritional components (isoflavone, protein, oil, and fatty acid) and antioxidant properties at the growth stage of different parts of soybean [glycine max (l.) merrill]. Food Sci Biotechnol. 2017 Apr;26(2):339-47. doi: 10.1007/s10068-017-0046-x, PMID 30263548.
2. Aldillah R. Analisis produksi dan konsumsi kedelai nasional [thesis]. Bogor (Bahasa): Institut Pertanian Bogor. Sekolah Pasca Sarjana; 2014. p. 127.
3. Bansal N, Parle M. Effect of soybean supplementation on the memory of alprazolam-induced amnesic mice. J Pharm Bioallied Sci. 2010 Jun;2(2):144-7. doi: 10.4103/0975-7406.67001, PMID 21814450.
4. Kusmardi K, Karenina V, Estuningtyas A, Tedjo A. Inhibition of cox-2 expression by lunasin-rich soybean extract on colorectal cancer. Int J App Pharm. 2019 Apr 11;Special Issue 6:116-21. doi: 10.22159/ijap.2019.v11s6.33572.
5. Sivamaruthi BS, Kesika P, Chaiyasut C. Influence of probiotic supplementation on climacteric symptoms in menopausal women-a mini review. Int J App Pharm. 2018 Nov;10(6):43-6. doi: 10.22159/ijap.2018v10i6.29156.
6. Satriyasa BK, Ni NB, Karmaya NM. Changes in male reproductive system induced by tempeh ethanol extract administered to rats from prenatal until weaning period of life.

Asian J Pharm Clin Res. 2018;11(5):109-13. doi: 10.22159/ajpcr.2018.v11i5.24173.
7. Georgetti SR, Casagrande R, Souza CRF, Oliveira WP, Fonseca MJV. Spray drying of the soybean extract: effects on chemical properties and antioxidant activity. LWT Food Sci Technol. 2008;41(8):1521-7. doi: 10.1016/j.lwt.2007.09.001.
8. Talele H, Rathod S, Pawar S, Raut A, Umarkar A. Soyabean as a neutraceutical a phytopharmacological. Res J Pharmacogn Phytochem. 2012;4(2):112-8.
9. Arnone B, Patrono E, Gasbarri A, Pompili A, Arnone B, Cavicchio A. Sex steroid hormone estrogen and cognition. Neurobiologia. 2011;74(2):121-38.
10. File SE, Jarrett N, Fluck E, Duffy R, Casey K, Wiseman H. Eating soya improves human memory. Psychopharmacol (Berl). 2001 Jul 6;157(4):430-6. doi: 10.1007/s002130100845, PMID 11605103.
11. Pankova S, Tsvetkova D. Role of phytoestrogens in prevention of osteoporosis. Int J Curr Pharm Res. 2015 Feb 28;7(2):1-6.
12. Muharam R, Budiman B, Rachman IA, Nirmalasari RA. Effect of daidzein 120-mg supplementation to menopausal symptoms and quality of life in non equol producer women. Asian J Pharm Clin Res. 2016 Aug 25;9(9):37-40. doi: 10.22159/ajpcr.2016.v9s3.14023.
13. Yang H, Jin GF, Ren DD, Luo S, Zhou TH. Neuro-protective mechanism of isoflavones on senes-cence-accelerated mice. Chin J Nat Med. 2010 Jul 20;8(4):280-4. doi: 10.3724/SP.J.1009.2010.00280.
14. Liu K. editor. Soybeans as functional foods and ingredients. AOC Press; 2004. p. 333.
15. Rostagno MA, Villares A, Guillamon E, Garcia lafuente A, Martinez JA. Sample preparation for the analysis of isoflavones from soybeans and soy foods. J Chromatogr A. 2009;1216(1):2-29. doi: 10.1016/j.chroma.2008.11.035, PMID 19041977.
16. Huang M, Liu D. Thin layer chromatography densitometric determination of soybean isoflavones in wild soybean (Glycine soja) seeds. Asian J Chem. 2012;24(3):1322-4.
17. McCue PP, Shetty K. Phenolic antioxidant mobilization during yogurt production from soymilk using kefir cultures. Process Biochemistry. 2005;40(5):1791-7. doi: 10.1016/j.procbio.2004.06.067.
18. Gunawan Puteri MDPT, Hassanein TR, Prabawati EK, Wijaya CH, Mutukumira AN. Sensory characteristics of seasoning powders from overripe tempeh, a solid state fermented soybean. Procedia Chem. 2015;14(Dec):263-9. doi: 10.1016/j.proche.2015.03.037.
19. Sivamaruthi BS, Chaiyasut C, Kesika P. Cosmeceutical importance of fermented plant extracts: a short review. Int J App Pharm. 2018 Nov;10(4):31-4. doi: 10.22159/ijap.2018v10i4.26355.
20. Ferreira MP, de Oliveira MCN, Mandarino JMG, da Silva JB, Ida EI, Carrao-Panizzi MC. Changes in the isoflavone profile and in the chemical composition of tempeh during processing and refrigeration. Pesqui Agropecu Bras. 2011;46(11):1555-61.
21. Day AJ, Dupont MS, Ridley S, Rhodes M, Rhodes MJC, Morgan MRA. Deglycosylation of flavonoid and isoflavonoid glycosides by human small intestine and liver beta-glucosidase activity. FEBS Lett. 1998 Aug;436(1):71-5. doi: 10.1016/s0014-5793(98)01101-6, PMID 9771896.
22. Murota K, Shimizu S, Miyamoto S, Izumi T, Obata A, Kikuchi M. Unique uptake and transport of isoflavone aglycones by human intestinal caco-2 cells: comparison of isoflavonoids and flavonoids. J Nutr. 2002 Apr;132(7):1956-61. doi: 10.1093/jn/132.7.1956, PMID 12097676.
23. Sulistyowati E, Martono S, Riyanto S, Lukitaningsih E. Development and validation for free aglycones daidzein and genistein in soybeans (Glycine max (L.) Merr.) using RP HPLC method. Int J App Pharm. 2019 Jan;11(2):138-42. doi: 10.22159/ijap.2019v11i2.31222.
24. Chaturvedi S, Luqman S, Khare SK. Facet of isoflavone, phenol and flavonoid content in soybean (Glycine max Merrill) varieties under dissimilar processing conditions. Ann Phytomed. 2014 Jun 30;3(1):50-5.
25. Nout MJR, Kiers JL. Tempe fermentation, innovation and functionality: update into the third millenium. J Appl Microbiol. 2005;98(4):789-805. doi: 10.1111/j.1365-2672.2004.02471.x, PMID 15752324.
26. Santoso. Teknologi pengolahan kedelai: teori dan praktek. Malang (Bahasa) Fak Pertanian Univ Widyagama; 2005. p. 37.
27. Rice Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Med. 1996;20(7):933-56. doi: 10.1016/0891-5849(95)02227-9, PMID 8743980.
28. Heim KE, Tagliaferro AR, Bobilya DJ. Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. J Nutr Biochem. 2002;13(10):572-84. doi: 10.1016/s0955-2863(02)00208-5, PMID 12550068.
29. Nan G, Shi J, Huang Y, Sun J, Lv J, Yang G. Dissociation constants and solubilities of daidzein and genistein in different solvents. J Chem Eng Data. 2014;59(4):1304-11. doi: 10.1021/je4010905.
30. Ahnan AD. Effects of tempeh fermentation on soy free and bound effects of tempeh fermentation on soy free and bound phenolics: release, transformation, and stimulated production phenolics: release, transformation, and stimulated production [doctoral dissertation]. MA: University of Massachusetts – Amherst. Department of Food Science; 253. p. 2020.
31. Andersen OM, Markham KR. editors. Flavonoids. Chemistry, biochemistry and applications. CRC Press Taylor & Francis Group; 2006. p. 1278.
32. Agume AS, Njintang NY, Mbofung CM. Effect of soaking and roasting on the physicochemical and pasting properties of soybean flour. Foods. 2017 Feb 9;6(2):12. doi: 10.3390/foods6020012, PMID 28231091.
33. da Silva LH, Celeghini RMS, Chang YK. Effect of the fermentation of whole soybean flour on the conversion of isoflavones from glycosides to aglycones. Food Chem. 2011 Mar 27;128(3):640-4. doi: 10.1016/j.foodchem.2011.03.079.
34. Borges CWC, Carrao Panizzi MC, Mandarino JMG, Silva JBd, Benedetti S, Ida EI. Contents and bioconversion of β-glycoside isoflavones to aglycones in the processing conditions of soybean tempeh. Pesq Agropec Bras. 2016;51(3):271-9. doi: 10.1590/S0100-204X2016000300009.
35. Setchell KDR, Brown NM, Zimmer Nechemias L, Brashear WT, Wolfe BE, Kirschner AS. Evidence for lack of absorption of soy isoflavone glycosides in humans, supporting the crucial role of intestinal metabolism for bioavailability. Am J Clin Nutr. 2002;76(2):447-53. doi: 10.1093/ajcn/76.2.447, PMID 12145021.
36. Aryanta WR. Traditional fermented foods in Indonesia. Japanese Journal of Lactic Acid Bacteria. 2000;10(2):90-102. doi: 10.4109/jslab1997.10.90.
37. Handoyo T, Morita N. Structural and functional properties of fermented soybean (tempeh) by using rhizopus oligosporus. Int J Food Prop. 2006;9(2):347-55. doi: 10.1080/10942910500224746.
38. Han BZ, Ma Y, Rombouts FM, Robert Nout MJR. Effects of temperature and relative humidity on growth and enzyme production by actinomucor elegans and rhizopus oligosporus during sufu pehtze preparation. Food Chem. 2003;81(1):27-34. doi: 10.1016/S0308-8146(02)00347-3.
39. Vital RJ, Bassinello PZ, Cruz QA, Carvalho RN, De Paiva JCM, Colombo AO. Production, quality, and acceptance of tempeh and white bean tempeh burgers. Foods. 2018 Aug 30;7(9):1-9. doi: 10.3390/foods7090136, PMID 30200205.
40. Taylor J, Ahmed IAM, Al-Juhaimi FY, Bekhit AEA. Consumers' perceptions and sensory properties of beef patty analogues. Foods. 2020 Jan 7;9(1):1-14. doi: 10.3390/foods9010063, PMID 31936123.
41. Rizal S, Kustyawati ME, Murhadi HU, Hasanudin U. The growth of yeast and fungi, the formation of β-glucan, and the antibacterial activities during soybean fermentation in producing tempeh. Int J Food Sci. 2021 Jan 27;2021:6676042. doi: 10.1155/2021/6676042, PMID 33575319.
42. Rostagno M, Manchon N, Guillamon E, Garcia Lafuente A, Villares A, Martines JA. Methods and techniques for the analysis of isoflavones in foods. In: Quintin TJ, editor. Chromatography types, techniques and methods. Nova Science Publishers, Inc; 2009. p. 1-43.