Food Research 6 (3) : 27 - 33 (June 2022)
Journal homepage: https://www.myfoodresearch.com

FOOD RESEARCH

FULL PAPER

The addition of lactic acid bacteria in the soybean soaking process of tempeh

Magdalena, S., Hogaputri, J.E, Yulandi, A. and *Yogiara, Y.

Faculty of Biotechnology, Atma Jaya Catholic University of Indonesia, Jl. Raya Cisauk, BSD City, Tangerang, Banten 15345, Indonesia

Article history:
Received: 5 May 2021
Received in revised form: 15 June 2021
Accepted: 5 September 2021
Available Online: 5 May 2022

Keywords:
Tempeh,
Soaking process,
Lactobacillus,
Sensory

DOI:
https://doi.org/10.26656/fr.2017.6(3).304

Abstract

Soaking is one of the important steps in tempeh production. Various microorganisms grow during the soaking process, which can affect the nutrition and quality of the tempeh. This study aimed to determine the effect of adding lactic acid bacteria (LAB) to the soaking process and determined the preference levels of the produced tempeh. In this research, *Lactobacillus plantarum* HPADA3 and *Lactobacillus fermentum* HPBD2 were added during the soaking of the soybeans. Each LAB had been evaluated for its hemolytic activity and potential virulence factor genes. The soaking duration was much shorter than was commonly done for tempeh production. The resulting tempeh was observed from their appearance and then underwent a sensory evaluation test. It was found that the shorter duration of soaking resulted in compact tempeh. The addition of LAB did not affect the fungal growth in tempeh fermentation. Overall, the bacteria population observed on agar media from the water and tempeh samples were significantly different. All produced tempeh was liked from an evaluation from semi-trained panellists. From all the tempeh variants, the tempeh with the addition of *Lactobacillus fermentum* HPBD2 was perceived as the most likeable and similar to commercial tempeh.

1. Introduction

Tempeh is a superior fermented food from Indonesia. Fermented food contains many beneficial compounds that are good for health. It is high in protein, vitamin B_{12}, antioxidants, and other bioactive compounds (Keuth and Bisping, 1993; Wu and Hasnah, 2018). Traditionally, tempeh is made from inoculated soybeans that are fermented to form a compact cake. The process of making tempeh includes boiling, dehulling, soaking, rinsing, drying, and fermentation with the inoculum (Mukhoyaroh, 2015).

In tempeh production, one of the most critical phases is the soaking process, where the dehulled soybeans are immersed in water for a length of time. Soaking is important because it improves the moisture content of the beans, supports the activity of microorganisms, and extracts saponin, which are the naturally occurring antimicrobial substances in soybeans. Fermentation takes place while soaking. Lactic acid bacteria (LAB) are one of the microorganisms found in the soaking process. It has the ability to produce acid, which plays a role in the acidification of the soaking process (Nout and Kiers, 2005). The soaking process influences the nutritive value and quality of the tempeh. For example, it supports the growth of beneficial organisms to produce vitamins (Radiarti and Sumarti, 2016) and inhibits the development of pathogenic bacteria (Usman *et al.,* 2018).

Even though the soaking process has a huge impact on the nutritive value of tempeh, the process is also time-consuming. This research proposes an alternative method by the addition of LAB to the soybeans during the tempeh soaking. LAB have been found to occur naturally in the tempeh making process and has a huge role in tempeh as probiotics (Touw, 2014). It produces acid (lactic acid and acetic acid) that can decrease the growth of spoilage and pathogenic microorganisms. Adding LAB in the soaking process was found to inhibit the growth of *Escherichia coli* (Winanti *et al.,* 2014). In this research, *Lactobacillus plantarum* HPADA3 and *Lactobacillus fermentum* HPBD2 were added to the soaking process of soybeans.

This research aimed to determine the effects of adding LAB in the soaking process of tempeh, and determine the preference level of the tempeh produced.

*Corresponding author.
Email: *yogiara@atmajaya.ac.id*

eISSN: 2550-2166
Published by Rynnye Lyan Resources

2. Materials and methods

2.1 Production and harvesting of biomass

One loop of each LAB was inoculated into MRSB and incubated at 37ºC until the OD was reached (around 16 hrs). OD was measured to reach biomass that was equal to 10^8 CFU/mL, with OD_{600} = 1.4 for *L. plantarum* HPADA3 and OD_{600} = 1.5 for *L. fermentum* HPBD2, then harvested through centrifugation at 5214 × *g* for 10 mins at 4°C (Hunaefi *et al*., 2012; Yadav *et al.,* 2016).

2.2 Safety assessments of lactic acid bacteria

Safety assessments were divided into hemolytic and virulence factors determination. For the hemolytic test, *L. plantarum* HPADA3 and *L. fermentum* HPBD2 were grown in MRSA media with the addition of 0.3% (w/v) $CaCO_3$ and then streaked onto blood agar plates containing 7% (v/v) sheep's blood (Yadav *et al*., 2016). The hemolysis activity of both LAB was evaluated after 24 hrs of incubation at 37ºC. The results were recorded by observing the clear zone (β-hemolysis), greenish zone or partial hydrolysis (α-hemolysis), or no reaction.

For virulence factors determination, both genomes from LAB were isolated using a Solg Genomic DNA Prep Kit (SolGent). Genes involved in the virulence factors were amplified using selected primers (Table 1). The virulence factors were *esp* for checking surface protein, *gelE* for gelatinase activity, *efaA* for surface A antigen, and *ace* for collagen adhesion (Sanchart *et al*., 2016).

The reaction conditions (25 µL) were as follows: 3 µL sample DNA, 8 µL PCR master mix (*Taq* polymerase, $MgCl_2$, DNTPs, and PCR buffer), 1 µL Primer F, 1 µL of Primer R, and 12 µL Nuclease Free Water. The PCR protocol was as follows: initial denaturation at 94ºC for 5 mins, denaturation at 94ºC for 1 min, annealing for 1 min (the temperature can be seen in Table 1), elongation at 72ºC for 1 min, final extension at 72ºC for 10 min, and then put on hold at 4ºC. The PCR was done in 30 cycles using a thermal cycler apparatus.

After amplifying, the resulting products were visualized using electrophoresis with 1.5% (w/v) agarose gel in TAE buffer 1×. A 100 bp DNA ladder was inserted in the left well of the gel, followed by each PCR product. Ethidium bromide was used for staining the bands. The bands were visualized with Biorad Universal Hood II Gel Doc System.

2.3 Tempeh production

Yellow soybeans (Pasar Modern BSD) were used as the raw materials to make tempeh. Each variation consisted of around 100 g of yellow soybeans. Soybeans were weighed, washed, boiled for 30 to 40 mins using underground water, rinsed, and dehulled by hands. The dehulled soybeans were given treatments as in Table 2. Each LAB in the treatments was added with the amount of 10^8 CFU/mL. After soaking, they were boiled for 30 to 40 mins, rinsed, and left to cool until they reached room temperature. Then, 1% (w/w) tempeh inoculum (Raprima^{TM}) was mixed into the soybeans (Winarno and Reddy, 1986). The inoculated soybeans were put into a perforated plastic and incubated for 30 hrs at 30ºC. The tempeh was observed from the front, back, and inside. Every variant was made in duplicate. For each soaking variation, water samples were taken before and after the soaking process.

Table 2. Various treatments in the soaking process

Tempeh variants	Treatments	Soaking time (hrs)
CT	Tempeh without any addition (control)	3
LF	*L. fermentum*	3
LFLP	*L. fermentum : L. plantarum* (1:1)	3
LP	*L. plantarum*	3

2.4 Enumeration of viable bacteria

Plate Count Agar (PCA), Eosin Methylene Blue (EMB), and deMan Rogosa Sharpe Agar (MRS agar) were used to grow the bacteria. PCA was used for growing mesophilic aerobic bacteria, EMB for *Enterobacteriaceae*, and MRS agar for lactic acid bacteria. For the test, 1 g of each tempeh variant was weighed and diluted with 9 mL sterile physiological saline in test tubes up to 1 mL. The diluted samples were spread on the agar media and observed (Radita *et al*., 2017). The water used after soaking was also spread on agar media. The results were counted in CFU/mL.

Table 1. Virulence factor genes for amplification

Primers	Sequences	Tm (ºC)	Amplicon size (bp)
esp	AGATTTCATCTTTGATTCTTGG AATTGATTCTTTAGCATCTGG	56	510
gelE	ACCCCGTATCATTGGTTT ACGCATTGCTTTTCCATC	52	419
efaA	GACAGACCCTCACGAATA AGTTCATCATGCTGTAGTA	54	735
ace	GAATTGAGCAAAAGTTCAATCG GTCTGTCTTTTCACTTGTTTC	56	1008

2.5 Sensory evaluation

A sensory evaluation test was then performed on the tempeh. It was divided into two parts: a simple overall differences test and a hedonic test. Approximately thirty-nine semi-trained panellists were asked to judge the sensory characteristics of the cooked tempeh. The overall differences test compared the control (commercial tempeh) to the tempeh with the addition of LAB, to determine which was the most similar to commercial tempeh. A hedonic test was also performed on the tempeh with LAB addition. All samples were cut to 2×2×1 cm and fried until the colour turned golden brown. The panellists rated the samples on a 7-point hedonic scale (1 = 'Strongly disliked'; 2 = 'Moderately disliked'; 3 = 'Slightly disliked'; 4 = 'Indifferent'; 5 = 'Slightly liked'; 6 = 'Moderately liked', and 7 = 'Strongly liked') for the following attributes: texture, odour, taste, aftertaste, and overall taste. The test was conducted in a sensory evaluation room with the assistance of red light. Water was provided for the panellists between samples. The analysis was done statistically with Statistical Package for the Social Sciences (SPSS) software with 5% probability.

3. Results and discussion

3.1 Safety of lactic acid bacteria isolates

Lactobacillus plantarum HPADA3 and *L. fermentum* HPBD2 were both isolated from the tempeh. From the assessments, the blood agar result turned out negative because there were no clear zones. Potential virulence factor genes were determined to be negative except for the *efaA* gene with a band size of 735 bp (Figure 1). Hemolytic activity was negative indicating they were not pathogenic (Yadav *et al.,* 2016). Potential virulence factor genes had one positive result; the *efaA* gene was responsible for surface A antigen (Sanchart *et al*., 2016) or cell wall adhesion which actually might help the LAB to colonize guts and showed its probiotic properties. This gene was also found in potential probiotics from Spanish sheep cheese by another study (Cebrián *et al.,* 2012). Another study also researched commercial probiotics in China that found the presence of potential virulence factors gene (Lei *et al*., 2015). A study on commonly used probiotics may contain virulence factor genes associated with colonization. The probiotics had been used for 20 years without any detrimental effects on the consumers. This suggested the presence of one or more virulence factors did not arbitrarily make the bacteria pathogenic (Franz *et al*., 2011).

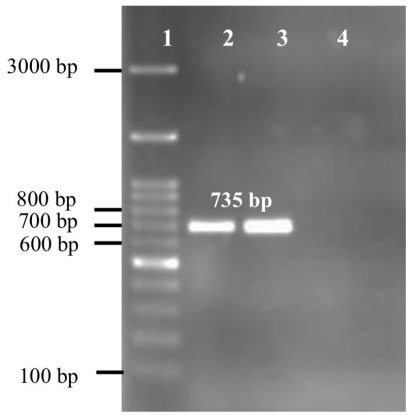

Figure 1. Agarose gel electrophoresis of *efaA* gene. 1: 100 bp DNA ladder, 2: *Lactobacillus plantarum* HPADA3, 3: *Lactobacillus fermentum* HPBD2, 4: negative control.

3.2 Tempeh production

Soybeans were soaked for 3 hrs with the addition of LAB then the water used for soaking was measured at the beginning and the end of the soaking period. The soaking duration was shorter than normally done for making tempeh where soybeans could be immersed in water for up to 30 hrs (Gunawan-Puteri *et al.,* 2019). The soaking water experienced a lowering of acidity level by around one degree of acidity (Table 3). pH number was comparable to a study where a soaking duration of 12 to 48 hrs could change pH from 6.5-7.0 to 4.5-5.5 or lowering two degrees of acidity (Mulyowidarso *et al*., 1989). Another study with 6 hrs of soaking changed the pH from 5 to 4.64. The changes in pH levels were caused by the activity of LAB which produced acid (Lumowa and Nurani, 2014). LAB was found in many stages of the tempeh making process and one of them was during the soaking process (Moreno *et al*., 2002)

All tempeh variants resulted in compact tempeh even though the pH was almost neutral, as seen in Figure 2. This result was similar to former studies. One study showed the effect of pH on *Rhizopus oligosporus* one of the main microorganisms for tempeh production. Interestingly, it was found that in the study replicating the tempeh fermentation condition, *R. oligosporus* was able to grow to pH 7.5 at 30ºC depending on the supporting CO_2 and $A_{w,}$ even if the growth was slow

Table 3. Acidity level of water before and after the soaking period

Tempeh variants	Treatments	pH	
		Before	After
CT	Without any addition	8.00	6.96
LF	*L. fermentum* HPBD2	7.58	6.48
LFLP	*L. fermentum* HPBD2 *: L. plantarum* HPADA3 (1:1)	7.53	6.41
LP	*L. plantarum* HPADA3	7.40	6.64

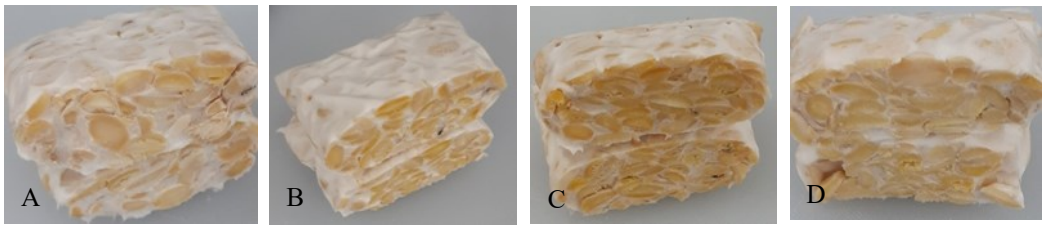

Figure 2. Appearance of (a) CT, (b) LF, (c) LFLP, (d) LP tempeh variants

(Sparringa *et al.,* 2002). In addition, Winanti (2014) also found that tempeh from Desa Batang in Banjar Province made with approximately pH 6 of soybeans resulted in compact tempeh.

A study had suggested LAB might affect fungal growth on tempeh, certain LAB strains had been known to inhibit fungal growth. The addition of LAB to aid fermentation in barley tempeh affected fungal growth depending on the types of bacteria and inoculation levels (Feng *et al*., 2005). Even so, the addition of LAB in this study resulted in tempeh with the same appearance as the CT tempeh. From this result, both LAB did not impact the growth of the inoculum, as the inoculum was able to grow normally and covered the soybeans.

3.3 Enumeration of viable bacteria

The water samples and tempeh samples were spread on PCA, EMB, and MRS agar media to determine the population of viable bacteria (Table 4). It was interesting to note that the morphology of bacteria growing from soak water was different on the PCA media. The CT had more variation of bacteria compared to all variants which were seemingly dominated by one type of bacteria. The morphology on the PCA plates appeared to be more uniform than the control, indicating the LAB was able to survive, affect the microbial composition, and dominate the water. A study about adding bacteria in seed soaking water indicated that the bacteria can be absorbed into the seed and increased the chance of survival against the physical condition and lower mesophilic bacteria count (Świeca *et al*., 2018). On EMB media, there was no metallic green sheen after soaking. The slight increase on EMB plates in the LF variant showed a different pattern than the CT. Even so, there were no metallic green sheen colonies found on EMB plates. There was the possibility that LAB did not inhibit other bacteria. The main mechanism of inhibition by LAB was organic acid but the inhibition was strain-specific and influenced by the amount of organic acid (Belicová *et al*., 2013).

Most water samples had significantly different bacteria populations than tempeh. Tempeh variants with the addition of LAB had higher bacteria on the MRS agar media compared to the CT. The presence of bacteria in tempeh was influenced by the second cooking process before fungal fermentation (Mulyowidarso *et al*., 1990) since it could have a lethal effect on bacteria (Nout and Kiers, 2005). Previous studies also had shown the impact of cooking soybeans on the number of bacteria. The number of bacteria was higher in the tempeh without a second cooking process as the population was reduced (Efriwati *et al*., 2013).

Heat-killed LAB might still play a beneficial role in food even after being inactivated and it may be beneficial to do further study. In one research, heat-killed *L. plantarum* could enforce the immune system shown by a lowered incidence of upper respiratory tract infection (Hirose *et al*., 2013). Another study about live and heat-killed *Lactobacillus rhamnosus* demonstrated that both had the ability to modulate the immune system by inducing cytokines. Even so, it should be noted that

Table 4. Enumeration of viable bacteria from the tempeh and water samples

Samples	Bacteria count (log CFU/mL)*		
	Total Viable Count	*Enterobacteriaceae*	Lactic Acid Bacteria
CT			
Soybean-soaked water	4.25±0.11[a]	3.42±0.18[a]	3.08±0.07[a]
Tempeh	7.33±0.23[b]	7.25±0.01[b]	3.73±0.16[a]
LF			
Soybean-soaked water	9.15±0.09[a]	3.96±0.04[a]	9.14±0.13[a]
Tempeh	6.89±0.01[b]	6.37±0.35[a]	6.41±0.07[b]
LFLP			
Soybean-soaked water	9.14±0.08[a]	3.19±0.12[a]	9.14±0.07[a]
Tempeh	6.50±0.02[b]	6.36±0.00[b]	6.52±0.09[b]
LP			
Soybean-soaked water	8.44±0.25[a]	3.91±0.08[a]	8.58±0.08[a]
Tempeh	7.42±0.33[a]	7.44±0.28[b]	6.39±0.11[b]

Values with different superscript within the same row are significantly different.

the ability was affected by the strain and genus of bacteria (Jorjão *et al.,* 2015). Tempe has been known to possess good health benefits if consumed. It is able to modulate human gut microbiota, but the effects may vary depending on the microorganisms (Soka *et al*., 2014).

3.4 Sensory characteristics and evaluation of produced tempeh

Sensory evaluation was conducted by 39 semi-trained panellists. For the first differentiation test, LF tempeh was perceived to be the most similar to commercial tempeh, followed by LFLP tempeh and LP tempeh. 14 panellists chose LF tempeh (35.90%), while 13 panellists chose LFLP tempeh (33.33%), and 12 panellists chose LP tempeh (30.77%). For further analysis, the likeability of all tempeh was determined through a hedonic test with the score showing that the texture and odour score between the tempeh was not significantly different. Taste, after taste, and overall testing were significantly different. The taste score of LF tempeh was significantly different compared to the other tempeh, receiving the highest score. The same pattern was also found on the aftertaste score. For overall testing, LF tempeh was significantly different to LP tempeh but not that different to LFLP tempeh. LFLP tempeh was also not too different from LP tempeh. From all the samples, LP tempeh received the least score on overall testing. The comparison of attributes can be seen in Figure 3.

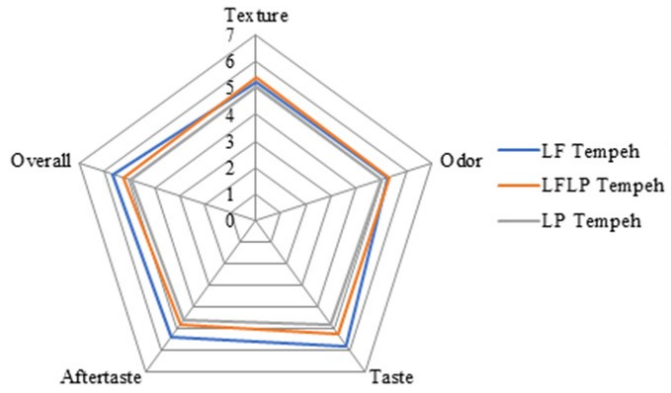

Figure 3. Hedonic test result of LF, LFLP, and LP tempeh

A sensory evaluation test was done using commercial tempeh and all tempeh variants. Of all the tempeh, the highest similarity with commercial tempeh was perceived on LF tempeh. LF tempeh was preferred significantly in terms of taste and after taste compared to LFLP and LP tempeh. Overall, it was significantly preferred over LP tempeh. In this study, the soybeans, water, processing method, and mould used were the same except for the use of bacteria in the soaking process. Differences in taste and after taste between the tempeh may be caused by different bacteria in the soaking stage. A similar case had been documented where bacteria affected a tempeh's flavour. Tempeh with a higher *Bacillus* sp. count had a more pronounced bitter taste compared to other tempeh (Barus *et al*., 2008).

In general, all the tempeh had fulfilled the quality requirement of texture, odour and colour from the National Standardization Agency of Indonesia (SNI 3144:2015) (BSN 2015). Odour and colour were considered normal. The attributes (odour, colour, and taste) were liked with an overall score ranging from 4.95 (LP tempeh) to 5.65 (LF tempeh). LF tempeh received positive remarks from panellists with a score between 5 and 6 (5.81) in taste, was slightly liked with a score of 5.41 in the aftertaste, and a score of 5.65 (almost 6 – moderately liked). Food can be claimed as probiotic food if it contained a minimum of 10^6 CFU/g viable bacteria (FAO/WHO, 2002). All the tempeh could be called probiotic tempeh since they all met this requirement. Although the number of LAB decreased after heat treatment, the killed bacteria might still have the potential to give health benefits to the immune system and should be studied (Hirose *et al*., 2013). All tempeh had a shorter soaking duration compared to conventional tempeh and LF tempeh was found to be the best variant. Tempeh as a superfood from Indonesia has increasingly gained a reputation in the worldwide community and has the potential to diversify food products and support local businesses (Astawan *et al*., 2017).

4. Conclusion

Lactobacillus fermentum HPBD2 and *L. plantarum* HPADA3 added to the soaking water grew without affecting the fungal growth. The addition *Lactobacillus* isolates increased the number of lactic acid bacteria in tempeh compared to control. All tempeh enriched with *Lactobacillus* in this study met the national standard requirement in texture, odour, and color. Tempeh added with *Lactobacillus fermentum* HPBD2 received better preference than tempeh added with *L. plantarum* HPADA3. The addition of LAB influenced the tempeh bacteria composition that might affect the health benefits of tempeh, while cell debris and metabolites produced may still have beneficial effects on health and was recommended for further study. Since all tempeh contained a minimum of 10^6 CFU/mL of viable bacteria on MRS agar, it can also be called probiotic tempeh.

Conflict of interest

The authors declare no conflict of interest.

References

Astawan, M., Wresdiyati, T. and Maknun, L. (2017).

Tempe: Sumber zat gizi dan komponen bioaktif untuk kesehatan. Bogor, Indoensia: Institut Pertanian Bogor (IPB). [In Bahasa Indonesia].

Belicová, A., Mikulášová, M. and Dušinský, R. (2013). Probiotic potential and safety properties of *Lactobacillus plantarum* from Slovak Bryndza cheese. *Biomedical Research*, 2013, 760298. https://doi.org/10.1155/2013/760298

BSN (Badan Standardisasi Nasional). (2015). Tempe kedelai: SNI 3144:2015. Jakarta, Indonesia: Badan Standardisasi Nasional. [In Bahasa Indonesia].

Barus, T., Suwanto, A., Wahyudi, A.T. and Wijaya, H. (2008). Role of bacteria in tempe bitter taste formation: microbiological and molecular biological analysis based on 16s rRNA gene. *Microbiology Indonesia*, 2(1), 17-21. https://doi.org/10.5454/mi.2.1.4

Cebrián, R., Baños, A., Valdivia, E., Pérez-pulido, R., Martínez-bueno, M. and Maqueda, M. (2012). Characterization of functional, safety, and probiotic properties of *Enterococcus faecalis* UGRA10, a new AS-48-producer strain. *Food Microbiology*, 30(1), 59-67. https://doi.org/10.1016/j.fm.2011.12.002

Efriwati, Suwanto, A., Rahayu, G. and Nuraida, L. (2013). Population dynamics of yeasts and lactic acid bacteria (LAB) during tempeh production. *Hayati Journal of Biosciences*, 20(2), 57-64. https://doi.org/10.4308/hjb.20.2.57

FAO/WHO (Food and Agriculture Organization/World Health Organization). (2002). Guidelines for evaluation of probiotics in food. London: Food and Agriculture Organization/World Health Organization.

Feng, X.M., Eriksson, A.R.B. and Schnürer, J. (2005). Growth of lactic acid bacteria and *Rhizopus oligosporus* during barley tempeh fermentation. *International Journal of Food Microbiology*, 104(3), 249-256. https://doi.org/10.1016/j.ijfoodmicro.2005.03.005

Franz, C.M.A.P., Huch, M., Abriouel, H., Holzapfel, W. and Gálvez, A. (2011). *Enterococci* as probiotics and their implications in food safety. *International Journal of Food Microbiol*ogy, 151(2), 125-140. https://doi.org/10.1016/j.ijfoodmicro.2011.08.014

Gunawan-Puteri M.D.P.T., Fortunata, S.A., Mursito, E. and Wijaya, C.H. (2019). Application of quick tempeh technology for production of overripe tempeh. *IOP Conference Series: Earth and Environmental Science*, 292, 012060. https://doi.org/10.1088/1755-1315/292/1/012060

Hirose, Y., Yamamoto, Y., Yoshikai, Y. and Murosaki, S. (2013). Oral intake of heat-killed *Lactobacillus plantarum* L-137 decreases the incidence of upper respiratory tract infection in healthy subjects with high levels of psychological stress. *Journal of Nutritional Science,* 2, E39. https://doi.org/10.1017/jns.2013.35

Hunaefi, D., Akumo, D.N., Riedel, H. and Smetanska, I. (2012). The effect of *Lactobacillus plantarum* ATCC 8014 and *Lactobacillus acidophilus* NCFM fermentation on antioxidant properties of selected in vitro sprout culture of *Orthosiphon aristatus* (Java Tea) as a model study. *Antioxidants,* 1(1), 4-32. https://doi.org/10.3390/antiox1010004

Jorjão, A.L., Oliveira, F.E., Vieira, M., Leão, P., Cláudio, C., Carvalho, T., Olavo, A., Jorge, C. and Oliveira, L.D. (2015). Live and heat-killed *Lactobacillus rhamnosus* ATCC 7469 may induce modulatory cytokines profiles on macrophages RAW 264.7. *Scientific World Journal*, 2015, 716749. https://doi.org/10.1155/2015/716749.

Keuth, S. and Bisping, B. (1993). Formation of vitamins by pure culture of tempeh moulds and bacteria during tempeh solid substrate fermentation. *Journal of Applied Bacteriology*, 75(5), 427-434. https://doi.org/10.1111/j.1365-2672.1993.tb02798.x

Lei, M., Dai X. and Liu, M. (2015). Biological characteristics and safety examination of five *Enterococcal* strains from probiotic products. *Journal of Food Safety*, 35(3), 1-12. https://doi.org/10.1111/jfs.12179

Lumowa, S.V.T. and Nurani, I. (2014). Pengaruh perendaman biji kedelai (*Glycine max,* L. Merr) dalam media perasan kulit nanas (*Ananas comosus* (Linn.) Merrill) terhadap kadar protein pada pembuatan tempe. *Jurnal EduBio Tropika*, 2(2), 187-250. [In Bahasa Indonesia].

Moreno, M.R.F., Leisner, J.J., Tee, L.K., Ley, C., Radu, S. and Rusul, G. (2002). Microbial analysis of Malaysian tempeh, and characterization of two bacteriocins produced by isolates of *Enterococcus faecium*. *Journal of Applied Microbiology*, 92(1), 147-157. http://doi.org/10.1046/j.1365-2672.2002.01509.x

Mukhoyaroh, H. (2015). Pengaruh jenis kedelai, waktu dan suhu pemeraman terhadap kandungan protein tempe kedelai. *Florea*, 2(2), 47-51. http://doi.org/10.25273/florea.v2i2.415 [In Bahasa Indonesia].

Mulyowidarso, R.K., Fleet, G.H. and Buckle, K.A. (1989). The microbial ecology of soybean soaking for tempe production. *International Journal of Food Microbiology*, 8(1), 35-46. http://doi.org/10.1016/0168-1605(89)90078-0

Mulyowidarso, R.K., Fleet, G.H. and Buckle, K.A. (1990). Association of bacteria with the fungal fermentation of soybean tempeh. *The Journal of Applied Bacteriology*, 68(1), 43-47. https://doi.org/10.1111/j.1365-2672.1990.tb02546.x

Nout, M.J.R. and Kiers, J.L. (2005). Tempeh fermentation, innovation and functionality: update into the third millennium. *Journal of Applied Microbiology*, 98(1), 789-805. https://doi.org/10.1111/j.1365-2672.2004.02471.x

Radiarti, A. and Sumarto. (2016). Analysis of physical properties, organoleptic properties, and nutritional values of tempeh from non-soybean legumes. *Jurnal Aplikasi Teknologi Pangan*, 5(1), 16-22. https://doi.org/10.17728/jatp.v5i1.32

Radita, R., Suwanto, A., Kurosawa, N., Wahyudi, A.T. and Rusmana, I. (2017). Metagenome analysis of tempeh production: where did the bacterial community in tempeh come from? *Malaysian Journal of Microbiology*, 13(4), 280-288. https://doi.org/10.21161/mjm.101417

Sanchart, C., Rattanaporn, O. and Haltrich, D. (2016). Technological and safety properties of newly isolated GABA-producing *Lactobacillus futsaii* strains. *Journal of Applied Microbiology*, 121(3), 734-745. https://doi.org/10.1111/jam.13168

Soka, S., Suwanto, A., Sajuthi, D. and Rusmana, I. (2014). Impact of tempeh supplementation on gut microbiota composition in Sprague-Dawley rats. *Research Journal of Microbiology*, 9(4), 189-198. https://doi.org/0.3923/m.2014.189.198

Sparringa, R.A., Kendall, M., Westby, A. and Owens, J.D. (2002). Effects of temperature, pH, water activity, and CO_2 concentration on growth of *Rhizopus oligosporus* NRRL 2710. *Journal of Applied Microbiology*, 92(1), 329-337. https://doi.org/10.1046/j.1365-2672.2002.01534.x

Świeca, M., Kordowska-Wiater, M., Pytka, M., Gawlik-Dziki, U., Bochnak, J., Złotek, U. and Baraniak, B. (2018). *Lactobacillus plantarum* 299V improves the microbiological quality of legume sprouts and effectively survives in these carriers during cold storage and *in vitro* digestion. *Public Library of Sciences* One, 13(11), 1- 13. https://doi.org/10.1371/journal.pone.0207793

Touw, K.T. (2014). Identification of Dominant Lactic Acid Bacteria during Tempeh Fermentation and Evaluation of Their Potential as A Probiotic. Bogor. Indonesia: Institut Pertanian Bogor. Thesis. [In Bahasa Indonesia].

Usman, N.A., Suradi, K. and Gumilar, J. (2018). Pengaruh konsentrasi asam laktat *Lactobacillus plantarum* dan *Lactobacillus casei* terhadap mutu mikrobiologi dan kimia mayonaise probiotik. *Jurnal Ilmu Ternak*, 18(2), 79-85. https://doi.org/10.1111/2049-632X.12145 [In Bahasa Indonesia].

Winanti, R., Bintari, S.H. and Mustikaningtyas, S.H.B. (2014). Studi observasi higienitas produk tempe berdasarkan metode inokulasi. *Unnes Journal of Life Sciences*, 3(1), 39-46.

Winarno, F.G. and Reddy, N.R. (1986). Tempe. In Reddy, N.R., Pierson, M.D., Salunkhe, D.K. (Eds). Legume based fermented foods. Boca Raton, USA: CRC Press.

Wu, S.K. and Hasnah, H. (2018). Nutrient contents in tempeh produced from five cottage industries in Selangor, Malaysia. *Malaysian Journal of Health Science*, 16(1), 1-6. https://doi.org/10.17576/jskm-2018-1601-20

Yadav, R., Puniya, A.K. and Shukla, P. (2016). Probiotic properties of *Lactobacillus plantarum* RYPR1 from an indigenous fermented beverage raabadi. *Frontiers in Microbiology*, 7, 1683. https://doi.org/10.3389/fmicb.2016.01683

