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Effect of tempeh wrapping on sensory evaluation and lactic acid bacteria profile

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Abstract

Tempeh is a fermented food made from soybeans and consumed daily due to its delicious flavour and high nutritional value. Traditionally, tempeh is wrapped in banana leaf, but nowadays, tempeh wrappers are often replaced with plastic because it is cheap and practical. However, high plastic utilization in daily life results in waste accumulation and causes environmental issues. Therefore, different types of leaves were explored as wrapping alternatives to reduce plastic utilization. This study aimed to determine the sensory characteristics of tempeh made with various wrappers and the lactic acid bacteria (LAB) characteristics of each tempeh. In this study, tempeh was wrapped in a teak leaf, bamboo leaf, lotus leaf, coconut leaf and plastic. Enumeration of total viable bacteria and LAB were not significantly different for all variants of the tempeh, except for the lotus leaf variant, which had a significantly lower number of LAB. The teak and coconut leaf variant tempeh were acceptable to panellists in the sensory evaluation test. The most preferred variant was the plastic-wrapped variant which had no significant difference from commercial tempeh. All LAB from the five types of tempeh were isolated to evaluate their haemolytic activity and antibiotic resistance. The result showed that all isolates exhibited negative haemolytic activity (gamma haemolysis). Most isolates (90.8%) were susceptible to chloramphenicol, while 80.3% of the isolates were resistant to vancomycin. The five isolates with the best characteristics were identified using the 16S rRNA gene. They were identified as *Enterococcus faecium*, *Staphylococcus gallinarum* and *Staphylococcus xylosus*.

1. Introduction

Tempeh is an Indonesian traditional fermented food that can be made from soybeans and other legumes. Generally, tempeh is fermented with *Rhizopus oryzae* or *Rhizopus oligosporus*. The tempeh-making process can be influenced by many factors such as temperature, humidity, fermentation time, and also wrapper (Astuti, 2009; Sayuti, 2015). Traditionally, tempeh is wrapped with various leaves such as banana and teak leaves. However, these days the tempeh wrapping is often replaced by perforated plastics. Plastics are used because they are cheap, easy to obtain and practical. High plastic utilization in human activities can result in waste accumulation that may cause environmental issues.

The increase in plastic waste has been a major concern in many countries, including Indonesia because more than 60% of total plastic solid waste is discarded in landfills, but only less than 10% of the waste is recycled (Lettieri and Al-Salem, 2011). Therefore, to reduce the utilization of plastic tempeh wrappers, different types of leaves were explored. In this study, several types of wrapping, such as a teak leaf, lotus leaf, bamboo leaf, and coconut leaf, were used to compare whether there were any differences in tempeh production. A previous study reported that different tempeh wrappers might show different sensory properties (Astuti, 2009). These leaves are commonly used as food wrappers in traditional food; they can be easily recycled and provide good aeration for the growth of *Rhizopus*, which is one of the main requirements of the fermentation process (Hidayat *et al.*, 2006).

Fermented food such as tempeh has an abundance of lactic acid bacteria (LAB). Lactic acid bacteria are microorganisms that can produce lactic acid as a fermentation product. Lactic acid bacteria are also known as probiotics. Probiotics have many advantages; they are beneficial to gastrointestinal health and can be

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used as food preservatives (Galvez *et al*., 2007; Mazahreh and Ershidat, 2009). During the tempeh soaking, the LAB pH will drop and inhibit the growth of other pathogenic bacteria. This study isolated and examined lactic acid bacteria from various tempeh. The objectives of this study were to determine the sensory characteristics (taste, aroma, texture) of tempeh made with various wrappers and determine the LAB characteristics in the tempeh.

2. Materials and methods

2.1 Materials preparation

Yellow soybeans were collected from the traditional market. The packaging used to wrap the tempeh in this study were plastics (zipper bags), teak leaves, dried lotus leaves, bamboo leaves and coconut leaves. Bamboo and coconut leaves were obtained from the online market, while teak and lotus leaves were collected from the Atma Jaya Catholic University of Indonesia. Lotus leaves were dried overnight in an oven at 37°C before use, while teak, bamboo and coconut leaves were used in fresh form.

2.2 Tempeh production

For tempeh production, about 100 g of white soybeans were used for each type of wrapper. Soybeans were washed and then boiled for 30 to 40 mins using groundwater. The boiled soybeans were then rinsed and dehulled by hand. The dehulled soybeans were soaked overnight in groundwater for bacterial fermentation. After the soaking process, soybeans were cooked for 30 to 40. Boiled soybeans were then rinsed and cooled. After this process, 0.2% (w/w) tempeh inoculum (Raprima, West Java, Indonesia) was inoculated into the soybeans (Magdalena *et al.,* 2021). Inoculated soybeans were wrapped in perforated plastic, teak leaves, dried lotus leaves, bamboo leaves and coconut leaves. The soybeans were then incubated for two days at 30°C. After incubation, the appearances of the tempeh were observed (front side, backside, inside). Every batch of production was carried out in duplicate.

2.3 Viable bacteria analysis

Viable bacteria were analyzed using plate count agar (PCA) to determine the number of mesophilic aerobic bacteria, Eosin methylene blue (EMB) agar to determine the number of *Enterobacteriaceae* (Barus *et al*., 2008), and de Man Rogosa Sharpe (MRS) agar supplemented with 0.3% (w/v) $CaCO_3$ to determine the total LAB. For sample analysis, about 1 g of each type of tempeh was added to 9 mL sterile physiological saline. Serial dilutions were made until 10^{-7}. From each dilution, 100 µL suspensions were spread on PCA, EMB and MRS agar. EMB and PCA media were incubated overnight at 37°C, while MRS agar was incubated for two days at 37° C aerobically. Bacteria that grew on the media were observed and counted manually (Feng *et al*., 2005). From the MRS agar, fifteen isolates from each variant of tempeh were picked for further analysis, such as haemolytic activity and antibiotic resistance activity assay.

2.4 Sensory evaluation

To determine the organoleptic properties of the tempeh, a sensory evaluation test was carried out on the selected tempeh variants. Semi-trained panellists conducted the sensory evaluation test with an age range between 20-22 years old. A hedonic test was conducted on the five samples of fried tempeh (teak leaf-wrapped tempeh, bamboo leaf-wrapped tempeh, coconut leaf-wrapped tempeh, plastic-wrapped tempeh and commercial tempeh (Rumah Tempe Indonesia, West Java, Indonesia). All samples were cut to 2 × 1 × 1 cm and fried until golden brown. About 35 panellists were asked to rate the samples based on the 7-point hedonic scale (1 = extremely dislike, 7 = extremely like) on five attributes: flavour, odour, texture, aftertaste, and overall. The sensory evaluation test was done with white light at room temperature in the sensory evaluation room. Panellists were asked to cleanse their palate with the water between each sample. The data collected were analyzed for statistical significance using Statistical Package for the Social Sciences (SPSS) software with a 5% probability (Paredes-Lopez *et al*., 1990).

2.5 Shelf life and moisture content assessment

The tempeh was evaluated for a few days to determine shelf life. They were placed at room temperature and observed every 6 hrs after 36 hrs of incubation until 72 hrs. The tempeh was also placed at a low temperature (4°C) and observed for two weeks. In both treatments, all tempeh variants were still wrapped in their packaging until the observation time was completed. The signs of deteriorated tempeh quality were black or yellow dots, a slimy texture and a change in aroma and texture (Sulistyono *et al*., 2016). Tempeh moisture content was assessed based on the procedure in SNI 3144:2105 (Badan Standardisasi Nasional (BSN), 2015). Metal plates were put in the oven at 100°C for 1 hr, then put in the desiccator for 20-30 mins, and their weight was measured. About 2 g of each tempeh variant were put on a plate and then put in the oven at 95-100°C for 5 hrs. After that, the plates were put in the desiccator for 30 mins and then put back in the oven for 1 hr. These steps were repeated until the weight of the sample was stable (weight difference ≤0.02 g). This method was conducted in duplicate. Tempeh moisture content was

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measured using this equation:

$$\text{Moisture content (\%)} = \frac{W1 - W2}{W1 - W0} \times 100\%$$

Where W1: weight of the sample before drying, W2: weight of the sample after drying and W0: weight of an empty plate

2.6 Haemolytic assay

LAB isolates that were isolated from the various tempeh were grown in de Man Rogosa Sharpe (MRS) agar medium at 37°C for 24 hrs, then streaked into blood agar and incubated at 37°C for 24 hrs (Oh and Jung, 2015). LAB isolate should show no sign of haemolytic activity.

2.7 Antibiotic resistance assay

Antibiotic resistance assay was performed by the disc diffusion method. LAB isolates from the various tempeh were tested against various antibiotics such as chloramphenicol (30 µg), vancomycin (30 µg), ampicillin (10 µg), enrofloxacin (5 µg) and kanamycin (30 µg). Isolates were refreshed in an MRS broth medium and incubated at 37°C for 24 hrs. Suspensions were diluted to a turbidity equivalent of 0.5 McFarland standard, then inoculated to MRS agar with three three-time continuous streaks using a sterile cotton swab. Antibiotic discs were placed on top of inoculated agar, and then incubated at 37°C for 24 hrs (Sujadmiko and Wikandari, 2017). The inhibition zone was measured and interpreted according to Clinical and Laboratory Standards Institute (CLSI) (2019) guidelines. Isolates with a zone of inhibition less than or equal to 14 mm are considered resistant. Those with a zone of inhibition between 15-19 mm are considered intermediate, while those with a zone of inhibition more than and equal to 20 mm are considered susceptible. This assay was conducted in duplicate.

2.8 Amplification and sequencing of pheS and 16S rRNA gene

The genome of the selected LAB was isolated using the Genomic DNA Prep Kit (SolGent, Daejeon, South Korea). Genomic DNA was added to the PCR mixture, which contained 10 pmol/µL of the primers. The total PCR reaction volume was 50 µL. In *the* gene amplification, primer *pheS*-21F and *pheS*-23R were used with conditions as described in Naser *et al.* (2005). Meanwhile, for 16S rRNA gene amplification, universal primers 63F and 1387R were used with conditions as described in Marchesi *et al.* (1998). The amplification result was visualized using agarose 1% gel electrophoresis at 90V for 1 hr, while the amplification product was sequenced and the data analyzed using BLASTn.

3. Results and discussion

3.1 Tempeh production

All tempeh variants were made with the same method, and the difference was the wrapping used for each variant. After 48 hrs of incubation, white mycelia knitted the beans thoroughly. The appearance of the tempeh in five different wrappings was the same, but for the lotus leaf tempeh, the leaf tends to stick on the tempeh's surface (Figure 1). Leaves have been used as tempeh wrappers because they give good aeration for the mold to grow. Results indicated that the usage of various leaf wrappers did not affect the growth of the tempeh starter. However, certain types of leaves may not be able to support the growth of mold, such as fresh lotus leaves. Fresh lotus leaf has a waxy surface (Ensikat *et al.,* 2011) which traps the water vapor inside the packaging and inhibits air circulation inside the wrapper, thus increasing the humidity and causing the soybeans to decay. A high level of humidity will also inhibit the growth of the mold because it favours the growth of spoilage bacteria (Shurtleff and Aoyagi, 2001).

3.2 Viable bacteria analysis

As shown in Table 1, the number of colonies in PCA ranged from 10^8 – 10^9 CFU/g. Tempeh wrapped in lotus leaf had the lowest number of colonies. The number of colonies between all tempeh variants were not significantly different. However, the number of bacteria between the lotus leaf and coconut leaf variants was significantly different (P > 0.05); where the coconut leaf variant was significantly higher than the lotus leaf variant. In EMB media, the number of colonies ranged from 10^8 – 10^9 CFU/g. Most of the colonies grown in the media for all tempeh variants were purple-black colonies, while there were also a few green colonies in the coconut and bamboo variants. The total of bacteria between the plastic, teak, and lotus leaf variants was not significantly different, while the total of bacteria between the bamboo and coconut leaf variants with the rest of the variants was significantly higher (P<0.05). The total of lactic acid bacteria between the five variants also ranged

Figure 1. Tempeh appearance of all variants: (a) plastic, (b) teak, (c) lotus, (d) bamboo, (e) coconut.

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from 10^8 – 10^9 CFU/g. The amount of LAB in the tempeh wrapped in the lotus leaf was significantly lower than in the rest of the tempeh variants. Moreover, the differences between the plastic, teak, bamboo, and coconut variants were not significantly different and tended to have a similar amount of LAB.

The results obtained were similar to a previous study by Han *et al.* (1999), where the total viable bacteria and *Enterobacteriaceae* in tempeh ranged between 10^8-10^9 CFU/g. Colonies grown in EMB indicated that *Enterobacteriaceae* such as *Klebsiella pneumoniae* which results in purple-black colonies, and *Citrobacter freundii,* which results in green metallic colonies, can be found in tempeh. Both of these bacteria have been reported to have the potential as vitamin B12 producers (Keuth and Bisping, 1993). In MRS agar, the number of lactic acid bacteria in the lotus leaf variant was significantly lower ($P < 0.05$) than in the others, while the other variants were not significantly different. Lactic acid bacteria from the genus *Lactobacillus*, *Leuconostoc,* and *Weissella* sp. have been reported to be found in tempeh (Yulandi *et al.*, 2020). During the soaking process, lactic acid bacteria have a role in producing organic acids such as lactic acid and acetic acid (Nout and Kiers, 2005), which inhibits the growth of spoilage and pathogenic bacteria (Feng *et al.*, 2005). Moreover, the number of bacteria in all variants ranged from log 8 to log 9 CFU/g, making them a good probiotic candidate as recommended by Food and Agriculture Organization (FAO)/World Health Organization (WHO) (2002).

The difference in total bacteria and lactic acid bacteria count might not be influenced by endophytic bacteria but might be because the lotus leaves had been dried. According to Santhirasegaram *et al*. (2016), the total number of mesophilic aerobic bacteria, lactic acid bacteria, moulds and yeasts was not affected by the variety of packaging but was mainly influenced by the fermentation process. Furthermore, Erdiansyah *et al.* (2022) found that the use of banana leaves and plastic as tempeh packaging did not provide a significant difference in the total number of lactic acid bacteria as measured by the total plate count method.

3.3 Sensory evaluation

About 35 panellists were asked to participate in the sensory evaluation test to rate the tempeh samples based on the hedonic scale to understand their preferences. Based on Table 1, commercial tempeh and the plastic-wrapped variant were not significantly different in all parameters (taste, aroma, texture, aftertaste and overall). The plastic-wrapped variant had the highest score in aroma, texture, and overall parameters, while commercial tempeh had the highest score in the taste and aftertaste parameters. For the aroma, texture, and aftertaste parameters, teak, bamboo, and coconut leaf variants were not significantly different from each other but significantly different from the plastic-wrapped variant and commercial tempeh. For the taste parameter, the bamboo leaf variant was not significantly different from the teak leaf variant but significantly different from the plastic-wrapped, coconut-leaf-wrapped, and commercial tempeh. The coconut leaf variant was also significantly different from the plastic-wrapped and commercial tempeh. Overall, the most preferred tempeh consecutively was the plastic-wrapped variant with a score of 5.49 (slightly liked), commercial tempeh with a score of 5.47 (slightly liked), coconut leaf variant with a score of 4.57 (neutral), and teak leaf variant with a score 3.97 (neutral). The bamboo leaf variant was the least preferred tempeh with a score of 3.71 (slightly disliked) (Table 2).

The difference in the panellists' acceptance may be due to taste preferences which can be influenced by many factors such as age, gender, habits and lifestyle. Food preferences may differ and change as we gain more experience with different kinds of food. It is also influenced by how we eat daily and how much exposure we get (Insel *et al.,* 2016). Panellists tend to prefer the plastic-wrapped variant compared to the other variants, probably because they are used to the sensory characteristics of a commercial product. Food made with plant-based wrappers usually has a unique aroma and flavour (Ng, 2015) that might not suit certain people. The difference in sensory characteristics and preference of tempeh samples wrapped in different packaging was also reported by Astuti (2009) and Umami *et al.* (2018),

Table 1. Enumeration of total viable bacteria, *Enterobacteriaceae*, and lactic acid bacteria.

Variant	Number of colonies (CFU/g)*		
	PCA	EMB	MRSA
Plastic	$2.31 \times 10^9 \pm 0.63^{ab}$	$3.10 \times 10^8 \pm 0.12^{a}$	$3.10 \times 10^8 \pm 0.09^{b}$
Teak	$1.43 \times 10^9 \pm 0.07^{ab}$	$1.60 \times 10^8 \pm 0.12^{a}$	$4.13 \times 10^9 \pm 0.43^{b}$
Lotus	$5.35 \times 10^8 \pm 0.06^{a}$	$4.45 \times 10^8 \pm 0.10^{a}$	$5.8 \times 10^8 \pm 0.04^{a}$
Bamboo	$4.96 \times 10^9 \pm 0.54^{ab}$	$6.73 \times 10^9 \pm 0.73^{b}$	$7.03 \times 10^9 \pm 0.01^{b}$
Coconut	$7.15 \times 10^9 \pm 0.18^{b}$	$4.46 \times 10^9 \pm 0.26^{b}$	$5.65 \times 10^8 \pm 0.07^{b}$

Values with different superscripts within the same column are statistically significantly different ($p<0.05$).

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Table 2. Sensory evaluation results for the five variants of tempeh.

Variant	Parameters*				
	Taste	Aroma	Texture	Aftertaste	Overall
Plastic	3.86±1.22[ab]	4.57±1.33[a]	4.66±1.24[a]	3.91±1.52[a]	3.97±1.38[a]
Teak	3.34±1.45[a]	4.37±1.59[a]	4.86±1.22[a]	3.49±1.40[a]	3.71±1.38[a]
Lotus	4.37±1.10[b]	4.34±1.10[a]	4.77±1.10[a]	4.09±1.07[a]	4.57±0.99[b]
Bamboo	5.31±1.39[c]	5.6±1.12[b]	5.74±1.01[b]	5.14±1.29[b]	5.49±1.07[c]
Coconut	5.41±1.35[c]	5.38±1.28[b]	5.62±1.42[b]	5.24±1.42[b]	5.47±1.36[c]

Values with different superscripts within the same column are statistically significantly different ($p<0.05$).

who found that tempeh wrapped in plastic packaging was more preferred because it has a stronger aroma typical to tempeh. Astuti (2009) also reported that tempeh wrapped in plastic has higher compactness than other packaging and thus was more preferred.

3.4 Shelf life and moisture content assessment

All variants of tempeh were put in two different conditions for shelf life assessment. In-room temperature, the surface of the tempeh wrapped in plastic became yellowish after three days, while tempeh made with bamboo leaves, lotus leaves, and teak leaves has a better appearance than plastic-wrapped tempeh. The texture and aroma of the bamboo, lotus and teak leaf tempeh variants were also better than the plastic variant. Furthermore, the tempeh wrapped in coconut leaves had the worst quality, as indicated by a dry, slimy texture, strong rotten odour, and moisture loss resulting in a dry texture. The shelf life of tempeh samples was also observed at low temperatures (4°C) for two weeks. There was not any significant change in their texture, aroma, and appearance for the first three days. The tempeh's quality started to deteriorate after one week, as indicated by yellowish mycelia and dry texture. The odour of the tempeh was also worsened. After two weeks, the surface of the tempeh became dry, but the inside was still soft. There was not any slimy texture observed. All tempeh variants displayed the same results, except for the mycelia of the plastic-wrapped variant, which was more yellowish than the others. The longer the tempeh was stored, the worse the quality (appearance, aroma, and texture) became. Commonly, the shelf life of fresh tempeh at room temperature is about two days and can last longer at low temperatures (Babu *et al.*, 2009; Astawan *et al.*, 2016).

The shelf life of all the tempeh variants was tested and showed that the tempeh wrapped with coconut leaves has the worst quality in aroma, texture, and overall appearance at room temperature. The coconut leaves were wrapped similarly to a *ketupat* with cavities on all sides; therefore, bacteria can quickly spoil the tempeh. Moreover, these contaminating bacteria can cause the tempeh's quality to decline faster than the other wrapping materials. Other than the coconut leaf variant, the plastic variant also displayed bad qualities compared to the teak, lotus, and bamboo leaf variants. The yellowish mycelia and rotten odour were quite strong, and there was a small amount of slime on the surface. The slimy, wet, and brown colour of the surface is caused by the growth of bacteria and yeasts at ambient temperature (Samson *et al.*, 1987). The rotten odour in tempeh is caused by ammonia resulting from the continuous fermentation of tempeh (Astawan *et al.*, 2016). Tempeh wrapped in leaves has better quality than tempeh in plastic wrappers because they have better air circulation than plastic wrapper, therefore having longer shelf life (Sulistyono *et al.*, 2016; Sari, 2018).

Additionally, all tempeh samples showed the same appearance at low temperatures, both inside and outside. There was not any slime observed. However, their texture was dry on the outside but soft on the inside. The tempeh's aroma also changed after two weeks of storage. These results indicated that tempeh stored at low temperatures has a longer shelf life than at room temperature. The quality of tempeh was preserved longer at low temperatures because bacteria growth was inhibited, and chemical changes were also delayed (Astawan *et al.*, 2016). Meanwhile, the moisture content for all the tempeh variants ranged from 57 to 59% (Table 3). Results showed that all tempeh variants have SNI 3144:2015 (BSN, 2015) requirements, which state that moisture content in tempeh should be below 65%.

Table 3. Moisture content of all tempeh variants.

Variant	Moisture Content (%)
Plastic	58
Teak leaf	59
Lotus leaf	57
Bamboo leaf	58
Coconut leaf	58

3.5 Haemolytic assay

All 76 isolates from the five variants of tempeh exhibited negative results (gamma-haemolysis). There was no haemolysis of blood cells in the media consisting of blood agar base and 10% sheep's blood. This result is

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similar to a previous study by Oh and Jung (2015). The absence of haemolytic activity is considered a safety prerequisite of FAO/WHO (2002).

3.6 Antibiotic resistance assay

The same isolates in the haemolytic assay were tested in this assay. Most of the isolates were resistant to vancomycin (80.3%) but susceptible/sensitive against chloramphenicol (90.8%), ampicillin (77.6%), and enrofloxacin (40.8%). Resistance was also shown to kanamycin (31.6%) and enrofloxacin (23.7%). Results are shown in Table 4.

Antibiotic resistance in bacteria has been studied widely due to its development rate and impact on global health. The presence of resistance may be intrinsic or acquired through gene transfer. Naturally occurring/ intrinsic resistance is not transferable horizontally and possesses no risk in non-pathogenic bacteria, while acquired resistance is present in bacteria and horizontally transferable (Mathur and Singh, 2005). Antibiotic resistance has been studied in some LAB (Kanak and Yilmaz, 2021; Guo *et al.*, 2017). In this study, most isolates were resistant to vancomycin, and several isolates were resistant to kanamycin but sensitive to chloramphenicol, ampicillin and enrofloxacin. This result is similar to Guo *et al.* (2017), where several strains of *Lactobacillus* sp. were resistant to vancomycin and kanamycin, but the resistance genes were not transferable among the bacteria. Vancomycin resistance is due to the alteration in the synthesis of peptidoglycan. This alteration may lead to peptidoglycan precursor production with low binding affinity to vancomycin or other glycopeptide drugs (Arthur *et al.*, 1996).

3.7 DNA isolation and sequencing

Based on the haemolytic and antibiotic resistance assay result, the selected isolates with the best characteristics were isolated B4, B10, B12, and B13 from the tempeh wrapped in bamboo leaves and T16 from the tempeh wrapped with coconut leaves. The *pheS* and 16S rRNA genes from selected isolates were amplified. The result for amplifying the *pheS* gene in five isolates was negative, while the result for the 16S rRNA gene was about 1300 bp. The 16S rRNA gene amplification results were sequenced and identified using BLASTn. Isolates B4 and B13 were identified as *S. gallinarum*, isolates B10 and B12 were identified as *E. faecium*, and isolate T16 was identified as *S. xylosus*. These bacteria have roles in fermented food as starter cultures. *Enterococcus faecium* belongs to lactic acid bacteria and can be found in fermented food, such as cheese (Erginkaya *et al.*, 2017) and fermented sausages (Franz *et al.*, 2011) to contribute to the ripening and aroma development of the product.

In the gene amplification, the result was negative. The *pheS* gene has been used as a reliable tool for identifying lactic acid bacteria from the genus *Lactobacillus*. However, it could not identify lactic acid bacteria from the genus *Enteroccocus* (Naser *et al.*, 2005; Naser *et al.*, 2007). Although isolates B10 and B12 were identified as lactic acid bacteria *E. faecium*, the primer used was not able to amplify the target gene because they may have different nucleotide sequences (Naser *et al.*, 2005). Enterococci resistance to vancomycin has also been reported by Erginkaya *et al.* (2017) in traditional Turkish fermented dairy products. It was also mentioned that *Enterococcus* spp. was susceptible to ampicillin and chloramphenicol. These results are similar to this study, where it was found that both isolates were resistant to vancomycin but susceptible to ampicillin and chloramphenicol. The use of *Enterococcus* in food as probiotics has been a controversial. They are favorable for their contribution to fermented foods' taste and flavour. However, some strains are pathogens for humans and animals (Santos *et al.*, 2015). Therefore, the application of *Enterococcus* sp. in food still needs further investigation.

Isolates B4, B13, and T16 were also isolated from MRS agar with $CaCO_3$ and produced clear zones. This clear zone indicated that *S. gallinarum* and *S. xylosus* can also produce acid (Kloos and George, 1991). Moreover, this also showed that both staphylococci could survive at low pH as they are found in several fermented foods. *Staphylococcus gallinarum* is found in soybean pastes such as miso (Kim *et al.*, 2010), while *S. xylosus* are naturally found in raw meat and fermented sausages. Some strains of both staphylococci are bacteriocin-producers that can be used as food additives (Laukova *et al.*, 2010; Medaglia *et al.*, 2010). Both *S. gallinarum* and *S. xylosus* are considered nonpathogenic *Staphylococcus*,

Table 4. Antibiotic resistance in LAB isolated from various tempeh.

Antibiotics	Number of isolates		
	Susceptible	Intermediate	Resistant
Vancomycin (VA)	0	15 (19.7%)	61 (80.3%)
Chloramphenicol (C)	69 (90.8%)	6 (7.9%)	1 (1.3%)
Ampicillin (AMP)	59 (77.6%)	10 (13.2%)	7 (9.2%)
Enrofloxacin (ENR)	31 (40.8%)	27 (35.5%)	18 (23.7%)
Kanamycin (K)	10 (13.2%)	42 (55.3%)	24 (31.6%)

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although a few strains have been found in infections. The discovery of *Staphylococcus* sp. in MRS agar was also reported in Prihanto *et al*. (2019), which isolated probiotic candidates from catfish.

4. Conclusion

Tempeh can be made with various plant wrappers. The utilization of various wrappers did not differ in appearance but may give different sensory characteristics in aroma and taste. In PCA media, the number of colonies was not significantly different. While in EMB, the number of bacteria in the coconut and bamboo leaf tempeh were significantly higher. The number of lactic acid bacteria in all variants was insignificantly different, except in the lotus leaf variant, which was significantly lower. Furthermore, the utilization of different wrappers also affected the shelf life of tempeh. For instance, the plastic-wrapped and coconut leaf variants tend to have shorter shelf life than the others. None of the lactic acid bacteria exhibited haemolytic activity. Most isolates were susceptible to chloramphenicol but resistant to vancomycin. Meanwhile, some isolates isolated from tempeh were identified as *S. gallinarum*, *S. xylosus* and *E. faecium*.

Conflict of interest

The authors declare no conflict of interest.

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