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Research Article

In vivo Evaluation of *Saccharomyces*-Modified Tempeh as Potential Prebiotic and Probiotic Food using *Mus musculus* as an Animal Model

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Abstract
Saccharomyces-modified tempeh (SM tempeh), which is produced by adding *Saccharomyces cerevisiae* during soybean fermentation, is considered to have the potential as a source of prebiotics. The research aims to determine the prebiotic activity score (PAS) of SM tempeh extract against the probiotics *S. cerevisiae* and *Lactobacillus casei*, as well as to evaluate the resistance of *S. cerevisiae* and *Escherichia coli* in the intestines of mice fed tempeh. The PAS evaluation was carried out using a factorial complete randomized block design with three replications and one-way ANOVA for data analysis followed by the least significant difference test (5%). Meanwhile, microbial survivability was carried out *in vivo* using male *Mus musculus* strain mice fed standard feed, and standard feed with tempeh extract supplementation. The results showed that the supplemetation of either SM or commercial tempeh extract to the growth media significantly affected on the microbial load of *S. cerevisiae, L. casei* and *E. coli*, but the concentrations of tempeh extract had no significant effect. Apart from that, the concentrations of tempeh extract had no effect on the PAS of *S. cerevisiae* and *L. casei*, meaning that it was able to promote the growth of probiotics in the amount added to the media in the range of 2–10%. In addition, the feeding type had a significant effect on the survival of *S. cerevisiae and E. coli* in the intestines. *S. cerevisiae* carried on SM tempeh was detected surviving in the mice intestine at a rate of 6.12 log CFU/g, indicating that the tempeh was a probiotic food. Most likely SM tempeh is a synbiotic food.

Keywords: *Mus musculus*, saccharomyces-modified tempeh, probiotic *S. cerevisiae*, prebiotic activity score, synergistic synbiotic food

1. INTRODUCTION

Probiotics and prebiotics are two elements that work together to regulate the microbiota in the human digestive system and improve the health of the host. When a food contains a combination of prebiotic and probiotic and there is a synergistic beneficial effect, then the food is considered a synbiotic food [1], either complimentary or synergistic symbiotic [2]. The previous term was a probiotic mixture formulation combined with a prebiotic which is used as food for the gut microbiota, while the second term is a combination of live probiotics in the formulation and selectively utilizes the prebiotic for the probiotic used in that formulation. For example, a mixture of *Lactobacillus sp* and its favorite food, lactose, which selectively supports the growth of *Lactobacillus sp*. In cooked tempeh, probiotics become either postbiotic or paraprobiotic because probiotics are deactivated and dead cells or their metabolite production help manage the immune system [3]. In the poultry industry, probiotics were used as supplements in an effort to minimize the use of antibiotics which could have an impact on disrupting the balance of intestinal microflora [4].

The ability of probiotics to colonize the intestinal mucosa, prevent pathogens and help improve the immune system, thereby increasing growth rate, improving health and enhancing the performance of broilers given probiotic supplements. Research on providing commercial probiotics to broiler chickens found a significant increase in average daily gain (ADG) and feed conversion ratio (FCR) [4]. Probiotic foods are foods that contain a number of probiotics and if consumed at least 1 million CFU/g of these probiotics are still alive in the human colon and provide health benefits [5]. Lactic acid bacteria, Bifidobacteria, *Bacillus subtilis*, *Saccharomyces cerevisiae*, and *Saccharomyces boulardii* are among the probiotics. *S. cerevisiae*, molecularly similar to *S. boulardii* is a probiotic yeast that is useful in the food industry due to its physiological properties and ability to use galactose [6]. Yet, this

Table 1. Growth of *L. casei*, *S. cerevisiae*, and *E. coli* for 24 h on SM tempeh extract.

Treatment	*L. casei* (Log CFU/g)			*S. cerevisiae* (Log CFU/g)			*E. coli* (Log CFU/g)		
	0 h	24 h	Δ	0 h	24 h	Δ	0 h	24 h	Δ
P1: media + glucose	8.02	8.98	0.96	7.70	8.50	0.8	5.90	5.23	-0.70
P2: media + inulin	8.03	9.17	1.15	7.12	8.55	0.85	5.93	4.44	-1.49
P3: media + 2% SM	8.03	8.53	0.51	7.21	8.45	0.75	5.89	4.46	-1.48
P4: media + 4% SM	8.03	8.91	0.88	7.10	8.79	1.10	5.93	4.58	-1.36
P5: media + 6% SM	8.30	9.09	0.79	7.31	8.7	1.04	5.90	4.63	-1.30
P6: media + 8% SM	8.03	8.06	0.61	7.20	8.36	0.66	5.93	4.85	-1.09
P7: media + 10% SM	8.07	8.85	0.78	7.11	8.74	1.04	5.93	4.73	-1.20

Note: media was MRS agar, PD agar, EMB Agar for *L. casei, S. cerevisiae, E. coli* respectively.

has not been widely applied to probiotic food products.

Saccharomyces-modified (SM) tempeh is tempeh produced by fermenting soybean using a mixed culture of *Rhizopus oligosporus* and *S. cerevisiae* [7]. In previous research, SM tempeh was found to carry *S. cerevisiae* in the amount of around 10^7–10^8 cell/g, contain β-glucans (0.13%), possess high vitamin B-12 (3.15 mg/100 g), have pleasant yeasty aroma dominated by alcohol, ester, and aromatic groups such as styrene, caryophyllene, phenol, and maltol, and be accepted by panelist with like scores [7]-[9]. SM tempeh was considered a probiotic food, if it was consumed *S. cerevisiae* would remain alive in the intestines and had a beneficial effect on the host. Dietary fiber foods that are resistant to digestive enzymes but selectively promote the growth of probiotic gut microbiota, are not utilized by enteric bacteria and provide health benefits to the host are prebiotic [10]. As a fiber-rich food containing probiotics, tempeh was considered a synbiotic candidate food. Galactooligosaccharides (GOS) in tempeh range from 21–34% (w/w), although it is lower than soybean oligosaccharides (47–53%) before fermentation it can help regulate the host's immune system [11]. Investigation on the bioactive oligosaccharides in tempeh against Enterotoxigenic *Escherichia coli* (ETEC) adhesion using yeast agglutination test indicated that the a high amount of oligosaccharides produced by bacterial tempeh increased the anti-adhesion of *E. coli* [12].

Other findings stated that high amount of tempeh diet increased the production of oligosaccharides, *n*-butyrate and propionate released by *Bifidobacterium* and *Lactobacillus sp.* in the cecum of mice used in experimental research, thereby reducing the concentration of immunoglobulin E. [13]. Sprague Dawley who was given tempeh for 28 d showed an increase in IgA production and IgA gene expression due to improved regulation of the gut microbiota [14]. Food components that have the potential to act as prebiotics can be determined using the Prebiotic Activity Score (PAS), which is a quantitative method for estimating the ability of a food component or the food itself to support the growth of probiotics but not support the growth of enteric bacteria [15]. In this study, SM tempeh flour was given to mice to ensure the survival of *S. cerevisiae* as a probiotic in tempeh. The study aims to determine the PAS of tempeh for the growth of probiotic *S. cerevisiae, L. casei,* and enteric bacteria *E. coli*, as well as determine the survivability of *S. cerevisiae* and *E. coli* in the intestine of mice.

2. MATERIALS AND METHODS

2.1. Materials

Soybean and commercial tempeh were purchased from the local market in Bandar Lampung. *R. oligosporus* and *S. cerevisiae* were isolated from commercial tempeh using potatoes dextrose agar (PDA) and from instant dried yeast using malt extract agar (MEA) respectively, deMan Rogosa Sharpe (MRS) agar for enumerating *L. casei*, and Eosin Methylene Blue Agar (EMBA) for enumerating *E. coli*. All chemicals used were analytical grade. *In vivo* test was carried out using animal model of *Mus musculus* strain Wistar, procured from the Palembang rat center (PRC,

Palembang, Indonesia). AIN 93-M standard mouse feed consisting of corn starch, casein, sucrose, L-cysteine, choline, soybean oil, CMC, vitamin mix, and mineral mix was mixed in the proper ratios [16].

2.2. Methods

2.2.1. Preparation of Modified Tempeh with Saccharomyces

The process of SM tempeh making followed a modified procedure [17]. A 100 g of soybeans were soaked in clean water overnight at room temperature. Next, they were washed and manually dehulled. The soybeans were then boiled at a ratio of 1:3 (soybeans:water) for 30 min, drained, and allowed to cool to room temperature. The next stage was inoculation with 0.4% (w/w) tempeh starter culture which is a mixture of *R. oligosporus* and *S. cerevisiaes* (Patent No. IDS000008337). The inoculated soybeans were then fermented at 32 °C for 40 h in polyethylene (PE) plastic bags with small pinhole aeration. The tempeh was then stored at refrigerated temperature until used for analysis.

2.2.2. Preparation of Tempeh Extract

Extraction of tempeh was conducted using the maceration method with modifications of the length of the freeze-drying process [18]. Tempeh powder was made by freeze-drying of fresh tempeh (aged 40 h), homogenizing it in water with a ratio of 1:10, then macerating at 120 rpm for 15 h at room temperature. The macerate was filtered, and the filtrate was concentrated using a rotary evaporator at 80 °C. The resulting macerate was stored in sterilized containers at refrigerated temperature until further use.

2.2.3. Prebiotic Analysis

Testing of the prebiotic ability to support probiotic growth was carried out using the described method [15]. Three microbial cultures (*S. cerevisiae, L. casei,* and *E. coli*) obtained were added to 7 test tubes, each containing 1 mL. Then, tempeh extract was added with the following treatments: P0, microbial growth medium without additional substrate; P1 (1% glucose); P2, (2% inulin); and P3, P4, P5, P6, and P7 were added with tempeh extract of 2, 4, 6, 8, and 10% w/v, respectively. Next, sterile growth media (MRSB/ PDB/NB) were added to each treatment up to 10 mL. The test tubes were lit and vortexed until homogeneous. They were then incubated for 1 d (bacteria) or 2 d (yeast) at 30 °C (yeast) or 37 °C (bacteria) aerobically, and the microbial culture density was determined.

The total microbial count was determined using the spread plate method, wherein 1 mL of sample from serial dilutions using physiological saline solution ranging from 10^7 to 10^9 for *L. casei*, *S. cerevisiae* (10^6–10^8), and *E. coli* (10^3–10^5) plated. Incubation was carried out at 37 °C for 24 h for *E. coli*, 37 °C for 24–48 h for *L. casei*, and at 30 °C for 48 h for *S. cerevisiae*. Colony counting was conducted using a colony counter according to International Commission on Microbiological Specifications for Foods (ICMSF) standards. The PAS calculation is expressed by the following

Table 2. Growth of *L. casei*, *S. cerevisiae*, and *E. coli* for 24 h on commercial tempeh extract.

Treatment	*L. casei* (Log CFU/g)			*S. cerevisiae* (Log CFU/g)			*E. coli* (Log CFU/g)		
	0 h	24 h	Δ	0 h	24 h	Δ	0 h	24 h	Δ
P1: media + glucose	7.04	7.54	0.50	6.94	7.83	0.89	4.57	4.94	0.37
P2: media + inulin	7.03	7.58	0.55	7.05	7.88	0.83	4.64	4.70	0.06
P3: media + 2% CT	7.04	7.51	0.47	7.10	7.78	0.68	4.26	4.62	0.36
P4: media + 4% CT	7.02	7.82	0.80	7.03	8.13	1.10	4.26	4.65	0.39
P5: media + 6% CT	7.03	8.07	1.04	7.10	8.07	0.97	4.26	4.72	0.08
P6: media + 8% CT	7.04	7.90	0.86	7.10	7.69	0.59	4.26	4.53	0.27
P7: media + 10% CT	7.02	7.85	0.83	7.05	8.07	1.02	4.26	4.60	0.34

Note: Data were the average of three replications. Media was MRS Agar, PD Agar, EMB Agar for *L. casei, S. cerevisiae, E. coli* respectively. CT = commercial tempeh.

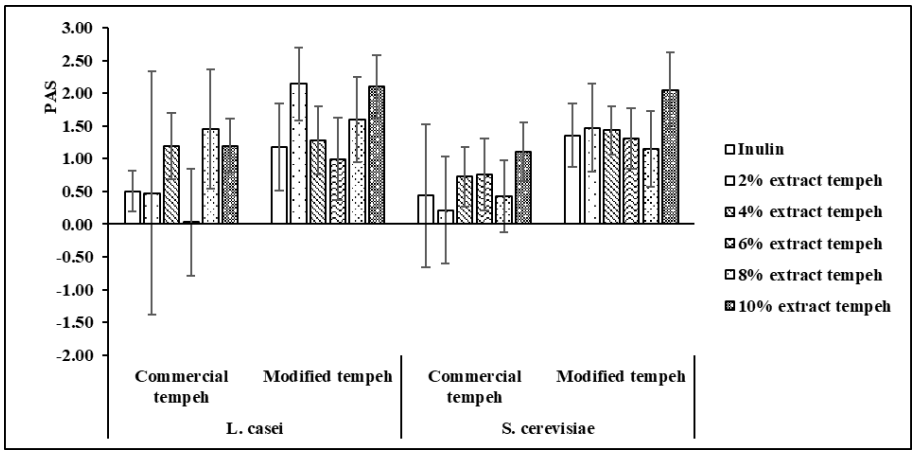

Figure 1. Prebiotic Activity Score (PAS) of modified tempeh extract and commercial tempeh. Data were the average of three replications with standard deviation.

Formula 1 [15]:

$$\text{PAS} = \frac{(Log\ P_{24} - Log\ P_0)_{Prebiotic}}{(Log\ P_{24} - Log\ P_0)_{Glucose}} - \frac{(Log\ E_{24} - Log\ E_0)_{Prebiotic}}{(Log\ E_{24} - Log\ E_0)_{Glucose}} \quad \textbf{(1)}$$

where log P_{24} is logarithm of probiotic bacterial growth on prebiotic culture and glucose after 24 h, log P_0 is logarithm of probiotic bacterial growth before 24 h (at 0 h) on prebiotic culture and glucose; log E_{24} is logarithm of enteric bacteria *E. coli* growth after 24 h on prebiotic culture and glucose, and log E_0 is logarithm of enteric bacteria *E. coli* growth before 24 h (at 0 h) on prebiotic culture and glucose.

2.2.4. In Vivo Analysis and Ethics Statement

In this experiment, mice were grouped into three groups and each was fed SM tempeh, commercial tempeh (without the addition of *S. cerevisiae*), and standard feed for mice (AIN 93-M). At the end of the experiment, mice were anesthetized using chloroform, and then the intestine and blood were taken for analysis. Three-month-old male Balb/C mice weighing 20–30 g were adapted under 22 °C in an experimental cage for 7 d, feeding with a standard diet and drinking water ad libitum according to the guidelines for laboratory animal use (Balitvet, Lampung, Indonesia). The difference in weight between mice in a group was < 10 g, and between mice groups was < 5 g. All groups were fed with a standard diet AIN93-M, consisting of corn starch 57, casein 14, soybean oil 4, CMC 5, water 5.07, mix mineral 3.5, mix vit 1, sucrose 10, L -cystine 0.18, choline 0.25. 100g-1 and of which was 351.6 calories,12% protein. After the feeding adaptation phase, the mice were weighed and randomized into 3 groups of five animals each and fed different experimental diets as follows. Group 1 for mice which were fed with a standard diet only diluted in 1 mL pure water. Group 2 for mice which were daily fed the SM tempeh powder 0.25 g/kg BW diluted in 1 mL pure water and standard diet. Group 3 for mice which were daily fed the commercial tempeh powder 0.25 g/kg BW diluted in 1 mL pure water and standard diet. At the end of the experiment, mice were anesthetized using ethylic-alcohol after 10 d of treatment to enumerate the yeast survivability and blood lipid profiles.

2.2.5. Enumeration of S. cerevisiae and E. coli in The Intestine of The Mice

Colon tissue samples were aseptically collected into a set of 5 mL sterile test tube containing 3 mL of 8.5 g/L NaCl, and diluted with dilution solution up to 10^{-6}. One mL of diluted sample was taken, spread plated method on MEA and EMB agar for enumerating *S. cerevisiae* and *E. coli* respectively, and incubated at 32°C for 24–48 h. The countable of *S. cerevisiae* in the colon indicated that the tempeh was a probiotic food. Meanwhile, the survival of *S. cerevisiae* was shown by a number of *E. coli* found in the intestines.

2.3. Experimental Design

The PAS study used a factorial complete randomized block design with various substrate compositions as the treatment factors (P) and was carried out three times. The treatment factor covered seven levels, where: P0 = medium without

supplementation of tempeh extract, P1 = media containing glucose (1%), P2 = media containing inulin (2%), and P3, P4, P5, P6, and P7= media supplemented with tempeh extract of 2, 4, 6, 8, and 10% w/v, respectively. The data were then analyzed using one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) test at the 5% level.

3. RESULTS AND DISCUSSIONS

3.1. Growth of Microorganism in The Medium Supplemented with SM Tempeh and Commercial Tempeh Extract

The growth of *L. casei, S. cerevisiae and E. coli* in a medium supplemented with SM tempeh extract and commercial tempeh extract respectively was presented in Tables 1 and 2. All the media containing glucose, inulin, and tempeh extract supplementation supported the growth of probiotics but were not utilized by *E. coli*. The available growth nutrient in tempeh may meet the needs of microbial growth. Meanwhile, bioactive components such as isoflavones aglycones, genistein, daidzein, and glycitein [17] in tempeh function as antioxidant, thereby inhibiting *E. coli*. On the other hand, glucose serves as a carbon source for the growth of all of the tested microorganisms [19].

3.2. PAS of SM Tempeh Extract on L. casei and S. cerevisiae

The PAS of SM tempeh extract and commercial tempeh extract (Figure 1) showed that there is no significant difference in various concentrations of commercial and SM tempeh ($p > 0.05$), this shows that all samples exhibit prebiotic properties. Positive prebiotic activity indicates that substrate containing tempeh extract can be utilized by *L. casei* and *S. cerevisiae* as effectively as glucose but not by other intestinal bacteria such as *E. coli*. This is possibly caused by the presence of GOS in tempeh which may not effectively be utilized by *E, coli* and can only be utilized by *S. cerevisiae and L. casei*. Zhang et al. reported that soybeans, the raw material for tempeh, contain GOS ranging from 47–53% which is a group of selective prebiotics to enhance the growth of probiotics [11]. The higher the PAS value, the better the tempeh extract is at supporting the growth of *L. casei* and *S. cerevisiae* while simultaneously suppressing the growth of *E. coli*. This aligns with Huebner et al., who stated that positive prebiotic activity values indicate that prebiotics can be utilized by probiotics as effectively as glucose, and this metabolism is specific to certain probiotics but not to other intestinal bacteria [20].

3.3. The Survivability of S. cerevisiae and E. coli in Mice Intestines

SM tempeh will meet the requirement as functional probiotic food if consumed, *S. cerevisiae* was found alive in minimum amounts ranging from 10^6–10^8 CFU/g in the intestine and the difference between its amount and the enteric bacteria was identified. *In vivo* assay using a mouse model was used to evaluate the survival S. *cerevisiae* in the mice intestine after the mice were fed SM tempeh, while enteric *E. coli* enumeration was to determine the effect of *S. cerevisiae* colonization. Previously, the viable *S. cerevisiae* of 8.62±0.14 Log CFU/g was identified in the SM tempeh powder at the time of administration to the mice. Dietary treatments consisting of SM tempeh and standard diet, commercial tempeh and standard diet, and standard diet only, were given to different groups of mice. After 10 d of feeding, the number of *S. cerevisiae* in the intestine of the mice was counted. ANOVA

Table 3. The viable *S. cerevisiae* and *E. coli* survived in the intestines of the mice fed diet treatments.

Diet treatments	*S. cerevisiae* (Log CFU/mL)	*E. coli* (Log CFU/mL)
AIN-93M (Group 1)	1.14 ±0.11^{a}	6.30 ±0.06^{c}
AIN-93M+SMT (Group 2)	6.12 ±0.05^{b}	5.87 ±0.05^{a}
AIN-93M+commercial tempeh (Group 3)	5.78± 0.04^{c}	6.11± 0.05^{b}

Note: The same letter followed the number in the same column in the table was non significantly different at LSD test ($\alpha \geq 0.05$). Data printed in Table were the average of the five replications.

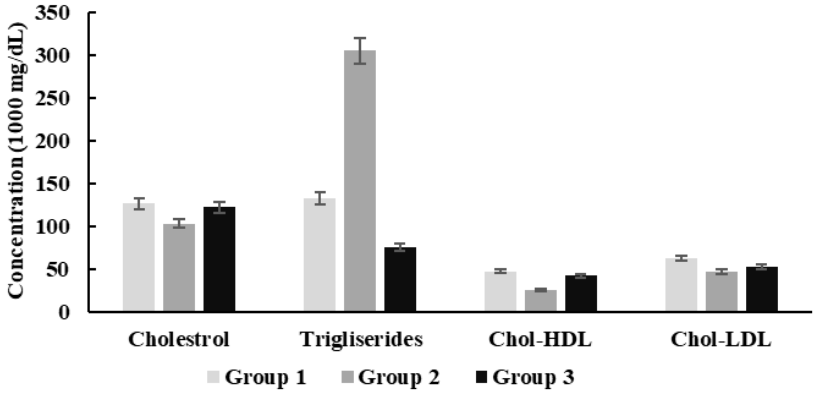

Figure 2 Lipid profile (cholesterol, triglycerides, cholesterol-HDL, cholesterol-LDL) of mice fed diet treatments. Group 1= mice fed AIN-93M, Group 2 = mice fed AIN 93M+SM) tempeh extract, Group 3 = mice fed AIN-93M+commercial tempeh extract. Data were the average of three replications with standard deviation.

showed that the diet treatments had a significant effect on the number of *S. cerevisiae* in the intestines of mice (Table 3). *S. cerevisiae* of SM tempeh (6.12±0.05 Log CFU/mL) was significantly higher than commercial tempeh *S. cerevisiae* (5.78±0.04 Log CFU/mL). This showed that there is high resistance to *S. cerevisiae* in the intestines of the mice after being fed for 10 d [20]. On the other hand, *S. cerevisiae* found in the intestines of mice fed commercial tempeh powder was 5.78±0.04 Log CFU/mL (less than 6 Log CFU/mL). Moreover, *S. cerevisiae* identified in the intestines of mice fed a standard diet (1.14±0.10 Log CFU/mL) was a yeast that naturally inhabit the intestines of mice because a standard diet did not contain *S. cerevisiae*. Roberfroid et al. observed that lactobacilli, streptococci, veillonellae, staphylococci, actinobacilli, and yeasts were the most dominant microbiota in the intestines [5]. Resistance of *S. cerevisiae* in the intestines of mice (Table 3) can prove that SM tempeh is a probiotic food. Foods that have the function of repairing intestinal epithelial cell walls, increasing adhesion to the intestinal mucosa, inhibiting intestinal pathogens, producing antimicrobial substances, and helping to modify the immune system, can be categorized as probiotic functional foods [21][22].

S. cerevisiae, having high cell wall protein, is acid tolerant, biofilm producer, and able to compete for nutrients, thereby its survival is high in the intestine. *S. cerevisiae* had the ability to stick to the surface of the intestine with the help of adhesin molecules through hydrophobic interaction [23]. After attaching to the intestinal surface, *S. cerevisiae* attaches to each other, proliferate and produce an extracellular matrix. The extracellular matrix consisting of carbohydrates, lipids proteins and amino acids exerts beneficial effects on *S. cerevisiae*, such as a source of nutrients, antimicrobial agents and cell adhesion. Chen et al. [24] and Kourelis et al. [25] found that the adhesive power of yeast cells was lower than that of bacteria due to the larger size of the yeast cells. However, the attachment mechanism was thought to be transient colonization, so the extracellular matrix might act more as a nutrient supply for probiotics or other gut microbes. Furthermore, *S. cerevisiae* is highly acid tolerant at the neutral to slightly acidic, they are able to gain energy from either aerobic through cellular respiration or anaerobic through the fermentation process [26], hence, they can grow under anaerobic conditions in the intestines. The number of *E. coli* found in the intestines of mice given SM tempeh was lower compared to commercial tempeh. Some properties of *S. cerevisiae* such as biofilm formation, acid production, and the cellular compound mycosine may contribute to *E. coli* inhibition [23]. This finding was in line with previous work concluding that increasing levels of *S. cerevisiae* in goat's milk kefir reduced *E. coli ATCC 25922* due to the antimicrobial activity exerted by *S. cerevisiae* [27].

Similarly, previous work investigating the survival ability of *E. coli O157:H7* against *S.cerevisiae* in a dynamic gastrointestinal model found that ethanol excreted by *S.cerevisiae* may be the main reason for high bacterial inactivation in the intestine [28].

3.4. Lipids Profile of Mice Fed Various Diet Consisting of Tempeh

Figure 2 shows the lipid profile of mice fed SM tempeh which had the lowest cholesterol, HDL-cholesterol, and LDL-cholesterol, but the highest triglyceride level. Meanwhile, mice fed commercial tempeh had the lowest triglyceride level. Vitetta et al. explained that probiotics modulate the non-hematogical intestinal interface [21]. However, Zavi˘sic et al. observed that supplementation of *Lactobacillus rhamnosus* Rosell 11 and *Lactobacillus helveticus* Rosell 53 at a concentration of 10^9 CFU/g reduced blood glucose levels and serum triglyceride levels in mice fed a high-fat high-sucrose diet, as a result of probiotic colonization leading to restoration of the intestinal barrier [29]. Meanwhile, the cholesterol-lowering effect exerted by *S. cerevisiae* may be due to bile salt hydrolase and the production of β-glucan and α-mannan [30]. Research on Sprague-Dawley rats fed a cholesterol-rich diet supplemented with β-glucan isolated from baker's yeast showed a reduction in total cholesterol and serum LDL cholesterol, but HDL cholesterol and triglycerides were not affected. There is a possible reason for the cholesterol-lowering effect of probiotics [31]. First is the incorporation of cholesterol in the phospholipid bilayer of the cell wall membrane of probiotics during growth. Generally, the cell membrane structure contains lipids and proteins, but in certain circumstances cholesterol is found combined in the phospholipid bilayer, this shows that cholesterol in the environment can be removed and incorporated into the cell membrane. Second is the conversion of cholesterol into coprostanol catalyzed by cholesterol reductase produced by intra and extracellular probiotics, which is directly excreted through feces. Another study conducted by Khorshidi et al. reported that the administration of monensin as a feed supplement to Zel sheep at any concentration showed a significant increase in blood metabolite products and commercial products [32]. Monensin is an antibiotic ionophore produced by *S. cinnamonensis* functioning to enhance animal feed efficiency and used as a growth promoter in ruminants and pigs.

4. CONCLUSIONS

The addition of either SM tempeh or commercial tempeh extract into the medium induces the growth of probiotic yeast *S. cerevisiae* and probiotic bacteria *L. casei*, but is not utilized *by E. coli*, thus showing potential as a prebiotic candidate. In addition, *S. cerevisiae* carried in SM tempeh was detected to survive in the mice intestine at an amount of 6.12 Log CFU/g, indicating that the tempeh can be considered as a probiotic food. Lipid measurements including cholesterol, triglycerides, cholesterol-HDL, and cholesterol-LDL showed that mice fed SM tempeh had higher triglycerides than other experimental mice. Meanwhile, all experimental mice showed no significant differences in cholesterol content. From these findings, it is likely that SM tempeh is said to be a synergistic synbiotic food. Synergistic synbiotics where probiotics and prebiotics are in the mix and work together to help improve the host's immune system. *S. cerevisiae* as a probiotic and tempeh itself as a prebiotic are two components of the mixture in SM tempeh.

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Research study design, M. E. K.; Performed the research, M. E. K. and T. S.; Research analysis help and advice, K. N. B. and S. R.; Data analysis, E. G. F.; Writing the original draft, M. E. K., E. G. F., and K. N. B.

Conflicts of Interest

The authors declare no conflict of interest.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the Health Research Ethics Committee, Faculty of Medicine, University of Lampung (No 1658/UN26.18/ PP.05.02.00/2023), in order to protect the rights and welfare of the health research subject and to guaranty that the research using biological material will carry out according to ethical, legal and other applicable.

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