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Pasteurization Effects on the Microbial Ecology of Functional Tempeh Developed from Black Rice Bran and Dual *Rhizopus* Strains

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Abstract

Background: *Black rice bran has the potential to be a functional food raw material due to its high content of bioactive compounds; however, it is susceptible to microbial contamination. This study aims to evaluate the effect of pasteurization on the microbiological quality of fermented rice bran tempeh using Rhizopus oligosporus and Rhizopus oryzae.* **Methodology:** *The study employed a comparative experimental approach, where fermented for 72 hours with R. oligosporus and R. oryzae, grown in PDA medium, with the addition of spore suspensions at 15% each (10^6 spores/mL). After fermentation, the bran was divided into two groups: one group was steamed for 10 minutes, while the other group was not steamed. Both groups were freeze-dried for 24 hours and then stored at -18 °C for analysis of changes in the profiles of total microbes, total fungi, total enterobacteria, and total spores. The raw material of rice bran was also analyzed microbiologically as a control. Microbiological data were analyzed using the Generalized Linear Model (GLM) and further evaluated by Duncan Multiple Range Test (DMRT) at a 5% significance level, as determined by SPSS 22.0 software.* **Findings:** *The results showed the pasteurization on bran tempeh products was effective in reducing the total number of microbes, moulds, Enterobacteriaceae, and spores compared to those without pasteurization. However, bran tempeh with R. oligosporus culture between pasteurized and unpasteurized did not show a significant decrease ($p>0.05$), except for the results of the analysis of the total number of moulds which showed a significant decrease ($p<0.05$), which was <1 log CFU/g (pasteurization) and 3.52 log CFU/g (without pasteurization). It can be concluded that the 10 minutes pasteurization process is effective in reducing the number of Enterobacteriaceae, which are indicators of polluting and pathogenic bacteria.* **Contribution:** *this treatment has potential to be applied on a home industry and Micro, Small and Medium Enterprises (MSME) scale to improve safety and extend the shelf life of fermented food products based on bran.*

Keywords: *Black Rice Bran; Fermentation; Microbial contamination; Pasteurization; Tempeh*

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INTRODUCTION

Fermentation is a food processing technique that has long been applied both traditionally and modernly. This fermentation process not only enhances the taste and shelf life of the product but also contributes to improving its nutritional value and bioavailability in the body. Tempeh is a typical Indonesian fermented product that is popular due to its high vegetable protein content and the presence of beneficial probiotic microbes, particularly moulds from the genus Rhizopus, such as *R. oryzae, R. stolonifer, R. microsporus,* dan *R. oligosporus* (Ardiani et al., 2024).

So far, tempeh has generally been made from soybeans as the primary raw material. However, along with the increasing need for functional foods and the utilization of agricultural waste, rice bran has begun to be considered as an alternative raw material. Rice bran is the outer layer of rice produced from the rice milling process. Rice bran, especially from black rice, has a high content of fiber, phenolic compounds, flavonoids, and anthocyanin activity, so it has the potential to provide health benefits to prevent degenerative diseases (Safrida et al., 2022; Budijanto et al., 2023; Budijanto et al., 2024). However, rice bran is also a material that is easily contaminated by environmental microbes, including pathogenic bacteria from the *Enterobacteriaceae* group, which can be an indicator of poor sanitation.

Tempeh fermentation typically utilizes *R. oligosporus* mould, but *R. oryzae* is also known to possess good fermentative abilities. Both play a role in forming the texture and aroma of tempeh, breaking down complex compounds in substrates such as proteins and polysaccharides into simpler compounds that are easier to digest, and releasing the bonds of bioactive compounds bound in the rice bran substrate (Ardiani et al., 2024; Safrida et al., 2022). Additionally, the presence of mould influences both the texture and the characteristic aroma of tempeh.However, each species has different growth characteristics and adaptability to substrates and fermentation conditions, which can also affect the final results microbiologically.

Based on prior studies, the optimal fermentation period for rice bran using culture of *R. oligosporus* and *R. oryzae* is 72 hours, as this duration has been shown to result in the highest levels of phenolic compounds, flavonoids, and antioxidant activity (Safrida et al., 2022). Therefore, a 72-hour fermentation period was applied in this study. Additionally, microbiological analysis of the product is necessary to assess the presence of indicator microorganisms that may indicate contamination. To reduce the number of initial contaminant microbes in rice bran, one method that can be used is pasteurization (steam) treatment. Pasteurization is a relatively mild and effective thermal method in reducing the number of pathogenic microorganisms without damaging critical bioactive compounds in rice bran (Tuncel, 2023). Pasteurization

process has an advantage over other thermal processes, such as sterilization or autoclaving, that may preserve nutritional and functional properties. It is more suitable for use in functional foods, especially in applications where fermented products are involved, such that the integrity of bioactive compounds must be maintained. Therefore, pasteurization was selected in this work to ensure microbial safety without affecting the useful compounds developed through fermentation.

Although rice bran-based tempeh has begun to be developed, scientific studies discussing the effect of pretreatments, such as steam, on the microbiological profile of this product are still limited. In particular, there have been few studies comparing the fermentation performance between *R. oligosporus* and *R. oryzae* on black rice bran substrates that have undergone pasteurization treatment. Therefore, this study is essential for evaluating how the effect of pasteurization influences the presence of microbial contaminants in black rice bran tempeh.

METHOD

Sample Collection

The study was conducted from August 2018 to September 2019 at the F-Technopark Laboratory of FATETA-IPB University, the Food Microbiology Laboratory of ITP-IPB, and the South-East Asia Food and Agricultural Science and Technology (SEAFAST) Center, IPB University. The research sample used was black rice of the Cempo Ireng variety, sourced from local farmers in Cigudeg, Bogor Regency, Indonesia.

Materials and Instruments

Additional research material included ingredients for fermenting black rice bran, namely *R. oryzae* inoculum (IPBCC 88010) and *R. oligosporus* inoculum isolated from Raprima (Lembaga Ilmu Pengetahuan Indonesia-LIPI). Then, Microbiological analysis required Potato Dextrose Agar (PDA), Butterfield's Phosphate-Buffered Diluent (BPB), Plate Count Agar (PCA), Buffered Peptone Water (BPW), Violet Red Bile Glucose Agar (VRBGA), and Nutrient Agar (NA) (Oxoid). The instruments used in this study for bran production included a husker machine (Yanmar, HW-60A, Japan), a rice polisher (N-70F type, Japan), and a 20-mesh sieve (Lion Star, Indonesia). Then, the fermentation setup consisted of an analytical balance (Chyo Balance, Japan), an autoclave (Hirayama, Japan), an incubator (Heraeus, Germany), a light microscope (Olympus, Japan), a hemocytometer, and glassware. For dehydration, a freeze dryer (Labconco, USA) was used. Microbiological safety analysis tools included a vortex mixer (Thermo Fisher, USA) and glassware.

Procedure Research

Black Rice Bran Sample

The black rice was ground using the Yanmar Rice Huller type HW-60A (Japan)] to obtain the brownish-black rough rice, which was then polished using Satake Rice type N-70 (Japan) for 90 s/200g, yielding two products: black rice bran and polished rice. After that, black rice bran is obtained by filtering through a 20-mesh screen to separate it from the broken grains.

***R. oryzae* Culture Preparation**

The culture of *R. oryzae* used to ferment rice bran is a fresh culture. The technique for refreshing the mould culture is as follows: one loop of culture from the original PDA medium is streaked onto the new PDA medium in a slanting manner. The latest PDA media is then incubated at 30 °C for 7 days. After the incubation process ends, the *R. oryzae* culture is suspended by adding 10 mL of sterile aquadest. The number of spores is then counted until it reaches exactly 10^6 spores/mL with a hemacytometer under a microscope. This spore suspension is ready to be inoculated onto the black rice bran substrate for the fermentation process stage (Nurtiana, 2018).

***R. oligosporus* Culture Preparation**

The Raprima tempeh solution is weighed as much as 1 g and then transferred into a test tube containing 9 mL of BPB diluent solution. This dilution is a 10^{-1} dilution stage; then, 1 mL is taken and placed into a new test tube containing 9 mL of sterile diluent solution for the 10^{-2} dilution. Serial dilutions were continued up to 10^{-7}. Each dilution of 10^{-5}, 10^{-6}, and 10^{-7} is taken as much as 1 mL and placed into a sterile, empty petri dish in duplicate. The petri dish filled with samples is supplemented with APDA media, which is made by mixing 100 mL of sterile PDA media (Oxoid, UK) with 1.4 mL of sterile 10% tartaric acid. The mixture is then incubated at 30°C for 3 days. After the incubation process ends, one loop culture is taken from the APDA media and streaked onto new PDA media slant agar as a culture preparation (Purwijantiningsih et al., 2019). The next step follows the *R. oryzae* culture preparation procedure above. The number of spores added to the fermentation medium has been subcultured and counted until it reaches exactly 10^6 spores/mL.

Black Rice Bran Fermentation

The rice bran fermentation method referred to Zulfafamy et al., (2018) with modification. The conditioning process started by preparing a rice bran substrate and adding distilled water at 50 % of the bran's weight to equilibrate the medium for optimal mould growth. Specifically, a 60 g portion of rice bran was transferred to a heat-resistant plastic bag, and 30 mL of distilled water was added, then mixed. Then, the rice bran was sterilized at 121°C for 15 min to reduce the risk of infection. After that, it was allowed to cool to 30 °C before inoculation. Each sample was inoculated with a spore suspension of *R. oryzae* and *R. oligosporus* at a concentration of 15% (10^6 spores/mL) relative to the rice bran weight, which was adjusted to 9 mL of suspension per 60 g of bran. Fermentation followed afterwards for 72 h at 30 °C under aerobic fermentation conditions. After fermentation, the rice bran was divided into two groups: one was steamed for 10 minutes (pasteurization) at 100 °C, while the other was left unsteamed. Both groups were freeze-dried for 24 hours and then stored at -18 °C for further analysis.

This study consisted of three treatment groups, namely: black bran fermented using *R. oryzae,* bran fermented using *R. oligosporus,* and bran without fermentation as a control. Each fermentation group was divided into two conditions: one with and one without pasteurization. At the same time, the control group did not undergo fermentation and pasteurization. Each treatment combination was carried out in two replications, resulting in a total of 10 experimental units analyzed to determine the total number of profiles of microbes, total fungi, total enterobacteria, and total spores.

Total Microbes

Total microbial analysis using the Total Plate Count (TPC) method. This analysis refers to the BAM (2001a) procedure. First, the dilution preparation was carried out by weighing a mashed sample of approximately 25 g into a heat-resistant, aseptically prepared plastic container. Then, 225 mL of BPB diluent solution was added. This dilution is a 10^{-1} dilution stage. The sample solution from the previous dilution was taken in an amount of 1 mL and placed into a test tube containing 9 mL of sterile diluent solution for dilution 10^{-2}. The exact steps to obtain dilutions 10^{-3} and 10^{-4} were followed. Then, 1 mL of sample solution was taken in each dilution and transferred into a petri dish in duplicate. PCA media was then added. The dish containing the sample and media was incubated for 48 ± 2 hours at a temperature of 35 ± 1 °C with the dish in an inverted position. Determining the total TPC value uses a calculation formula referring to BAM (2001a):

$$N = \frac{\Sigma C}{(1 \times n_1) + (0.1 \times n_2) \times d}$$

Description:

N: Number of colonies per ml or per gram of product
ΣC: Number of colonies counted (TPC: 25-250, mould: 10-150)
n1: Number of plates in the first dilution
n2: Number of plates in the second dilution
d: First dilution counted

Total mould

Total mould analysis refers to the BAM (2001b) procedure. This analysis begins by weighing 25 g of the finely ground sample, then suspending it in 225 mL of BPB diluent solution (10^{-1} dilution). The sample solution is taken in an amount of up to 1 mL and placed into a test tube containing 9 mL of sterile diluent solution, resulting in a 10^{-2} dilution. The same steps are taken to obtain a dilution series up to 10^{-4}, and then plating is carried out using the pour plate method. First, 1 mL of sample suspension is taken at each dilution to be transferred into a sterile petri dish in duplicate. The petri dish filled with the sample is supplemented with APDA media, which is made by mixing 100 ml of sterile PDA media (Oxoid, UK) with 1.4 ml of sterile 10 % Tartaric acid. The mixture is then incubated at 30 °C for 3 days. The calculation utilizes the total TPC value formula, which can be found in the total microbial analysis.

Total *Enterobacteriaceae*

In the analysis of *Enterobacteriaceae*, as per Jersek (2017), the sample was weighed up to 10 g in a heat-resistant, aseptically prepared plastic container and then suspended in 90 mL of BPW diluent solution. This dilution is a 10^{-1} dilution stage. The sample solution from the previous dilution was taken in an amount of 1 mL and transferred to a test tube containing 9 mL of BPW and then homogenized. This dilution is a 10^{-2} dilution. The same steps were carried out until a dilution of 10^{-4} was obtained. Then, 1 mL was taken from each dilution and transferred to each petri dish, with VRBGA selective medium added. The dish containing samples and media was incubated for 24 ± 2 hours at 30 °C. *Enterobacteriaceae* colonies on VRBGA media

appeared pink to red or purple, with or without a halo of light around them. Colonies are counted using the total TPC value formula, as shown in the total microbial analysis.

Total Spore

In total spores, the spores analyzed are bacterial spores refers to the Harrigan (1998) procedure. The total spore dilution procedure is identical to the 10^{-1} dilution procedure used in total bacterial analysis. The sample, diluted 10^{-1}, was then heated to 150 mL using an autoclave for 10 minutes at a temperature of 80 °C. The heated sample, taken in an amount of up to 1 mL, was then diluted by adding 9 mL of BPB diluent solution to achieve a 10^{-2} dilution. The same steps are carried out to obtain a 10^{-3} dilution, and then 1 mL of each dilution is inoculated into a sterile petri dish in duplicate, with NA media added. The dish containing samples and media is incubated for 48 hours at 30 °C. Observe the presence or absence of colony growth and count the colonies that grow on each NA medium using the total TPC determination formula, as outlined in the total microbial analysis.

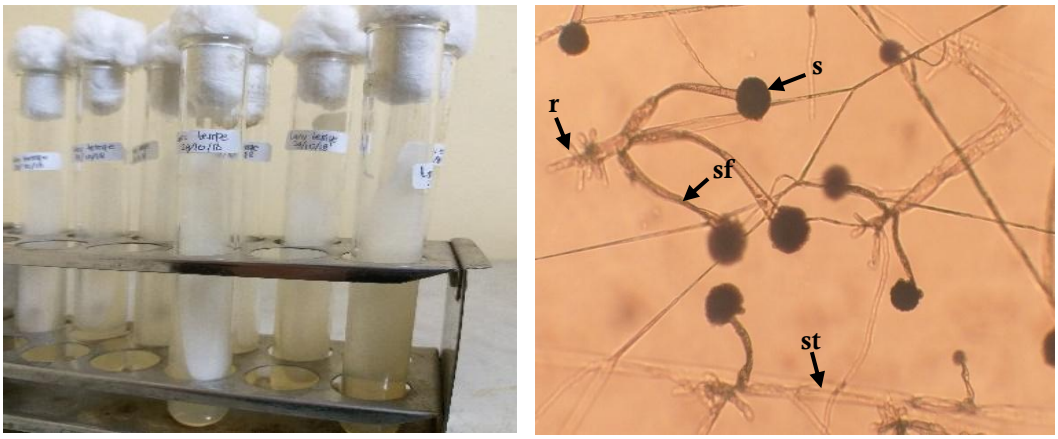
Data analysis

The data from the analysis were processed using Microsoft Excel software. The microbiology results were followed up with Generalized Linear Model (GLM) analysis and further tested using the Duncan Multiple Range Test (DMRT). The significance value was determined based on a 5 % level of significance using SPSS 22.0 software (Chicago, IL, USA).

RESULT AND DISCUSSION

Microbiological analysis of bran tempeh aims to assess the microbial quality and sanitation indicators, which can indicate food safety in the product. In this study, an examination of the mould culture of *R. oligosporus* and *R. oryzae* was also carried out to ensure that the cultures used were pure cultures and free from contaminants. Examination of this culture was conducted microscopically using the slide culture method, which involved observing the mould's morphology under a microscope. The results of observations of the mould *R. oligosporus* are presented in Figure 1, and the mould *R. oryzae* is presented in Figure 2.

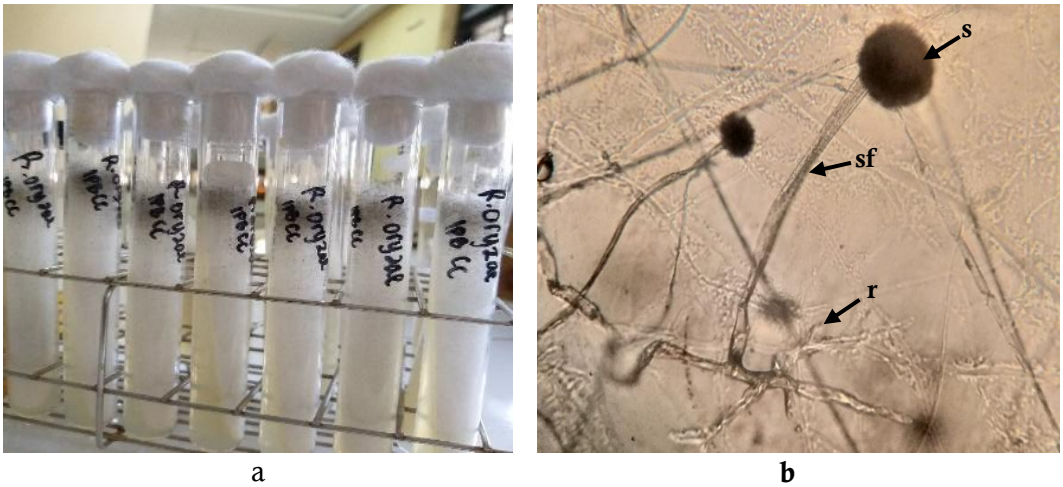
Based on the results of microscopic identification, Figure 1 shows the characteristics of *R. oligosporus*, namely the shape of the sporangium, which is round and brownish black, single and short sporangiophores, and has short hyphae that are not septate with short rhizoids (1b). It is by what was reported by several researchers that the *R. oligoporus* mould has a round and brownish-black sporangiospore and sporangium shape, equipped with short, non-septate hyphae, short and single sporangiophores, and semi-round columella and short rhizoids (A'yun & Janah, 2022; Rodhibilah et al., 2023; Ardiani et al., 2024). Thus, it shows that the mould species in the RAPRIMA solution is only a pure culture of *R. oligosporus*. It is based on the report by Rodhibilah et al., (2023), who identified isolates from the results of RAPRIMA solution isolation. The type of mould obtained is *R. oligosporus*.



a b

Figure 1. Determination of *R. oligosporus* from Raprima tempeh inoculum. Isolation in PDA agar slant after purification (a), microscopic results of the morphology of *R. oligosporus* mould at 100x microscope magnification (b), with descriptions of sporangium (s), sporangiophore (sf), rhizoid (r), stolon (st)

Meanwhile, the morphological identification results of the *R. oryzae* mould in Figure 2 show that the colonies formed on the PDA slant agar after purification are greyish-white with a smooth texture (2a). Furthermore, microscopic observations reveal a round sporangium shape, a thorny wall with a blackish-brown colour, and a single sporangiophore with non-septate hyphae (2b). The results of the same study were also reported by Ardiani et al., (2024), who noted that *R. oryzae* macroscopically exhibits a colony with a smooth texture and a greyish-white appearance. Then, microscopically, this mould has a round sporangium with a thorny wall, a single sporangiophore, non-septate hyphae, and some are in groups of up to five, forming a fork-like branching structure with smooth walls. Based on these characteristics, it is clear (Figures 1 and 2) that the culture used in this study is a pure culture and free from contaminants.



a **b**

Figure 2. Determination of *R. oryzae* from IPBCC 88010. Isolation in PDA agar slant after purification (a), microscopic results of the morphology of *R. Oryzae* mould at 400x microscope magnification (b), with descriptions of sporangium (s), sporangiophore (sf), rhizoid (r), stolon (st)

Table 1. Microbiological Analysis Results of Bran Tempeh

Microorganism	Raw Material (log CFU/g)	Bran Tempeh			
		Pasteurized (log CFU/g)		Unpasteurized (log CFU/g)	
		R.oligosporus	***R.oryzae***	***R.oligosporus***	***R.oryzae***
Total microbes	4.96±0.07^{c}	2.46±0.68^{ab}	1.45±0.64^{a}	3.30±0.33^{b}	3.46±0.47^{b}
Total moulds	5.27±0.10^{c}	<1±0.00^{a}	<1±0.00^{a}	3.52±0.33^{b}	3.64±0.10^{b}
Total *Enterobacteria*	4.79±0.05^{b}	<1±0.00^{a}	<1±0.00^{a}	1.39±0.55^{a}	<1±0.00^{a}
Total spores	4.10±0.28^{c}	1.87 ±0.98^{ab}	<1±0.00^{a}	1.69±0.06^{ab}	2.63±0.26^{b}

Data presented were mean± standard deviation. Different superscripts on the same column and row showed significant differences at the 5% test level.

Meanwhile, the results of the microbiological analysis of bran tempeh, presented in Table 1, show that the total microbes, moulds, *Enterobacteriaceae*, and spores contained in bran tempeh were significantly reduced ($p < 0.05$) compared to the raw bran materials. The reduction in the number of microbes, moulds, *Enterobacteriaceae*, and spores is due to the sterilization of the bran before fermentation. According to Yastrebov (2025), the moist heat sterilization process (autoclave) can eliminate and destroy microorganisms, including viruses, fungi, bacteria, and bacterial spores, which are different from the spores of molds such as *R. oligosporus* dan *R. oryzae* that are used in the fermentation process. Thereby preventing infection, contamination, and spoilage in food. It is also supported by the results of macroscopic observations (Figure 3), which show a reduction in the number of colonies on the agar medium from raw bran materials, whether pasteurized or unpasteurized, in tempeh.

Bran tempeh pasteurized for 10 minutes showed lower total number of microbes, moulds, *Enterobacteriaceae*, and spores compared to unsterilized bran tempeh, but in bran tempeh with *R. oligosporus* culture between pasteurized and unpasteurized did not show a significant reduction ($p>0.05$), except for the results of the total mould analysis showing a significant reduction ($p<0.05$) (Table 1). Meanwhile, the results of the total spore analysis in bran tempeh with *R. oligosporus* culture did not show a decrease in the number of spores after the pasteurization process. It is suspected that spores originating from the raw materials did not all die during the sterilisation or pasteurisation process.

The resistance of spores to high temperatures has been widely reported in various studies. Bacterial spores, such as those from the genera *Bacillus* and *Clostridium*, possess a multilayer structure (Ca-DPA) and contain protective compounds, including calcium dipicolinate and SASPs (Small Acid-Soluble Spore Proteins), which render them highly resistant to heat (Setlow, 2014).

In the context of pasteurization, temperature and exposure time are two critical factors that affect the ability to inactivate spores. Pasteurization uses a temperature of < 100°C for a particular duration of time, so it is only effective in killing vegetative microorganisms without destroying spores. The cortex and coat layers, which protect the spores, are not permanently damaged at lower temperatures; therefore, the spores remain viable and can germinate again when environmental conditions become favourable (Wen et al., 2022). This is why, in bran tempeh made with pasteurized

R. oligosporus culture, there is no significant difference ($p > 0.05$) compared to the treatment without pasteurization.

Additionally, contamination may also occur through contact with ambient air or during the fermentation process. However, the pasteurisation process on bran fermented by *R. oligosporus*, although it did not reduce the number of spores, was effective in reducing the number of microbes, moulds, and *Enterobacteriaceae* compared to bran fermented without pasteurisation. It was reinforced by the results of macroscopic observations, which showed that several microbial colonies remained in the media after treating bran tempeh without pasteurisation (Figure 3A-3D).

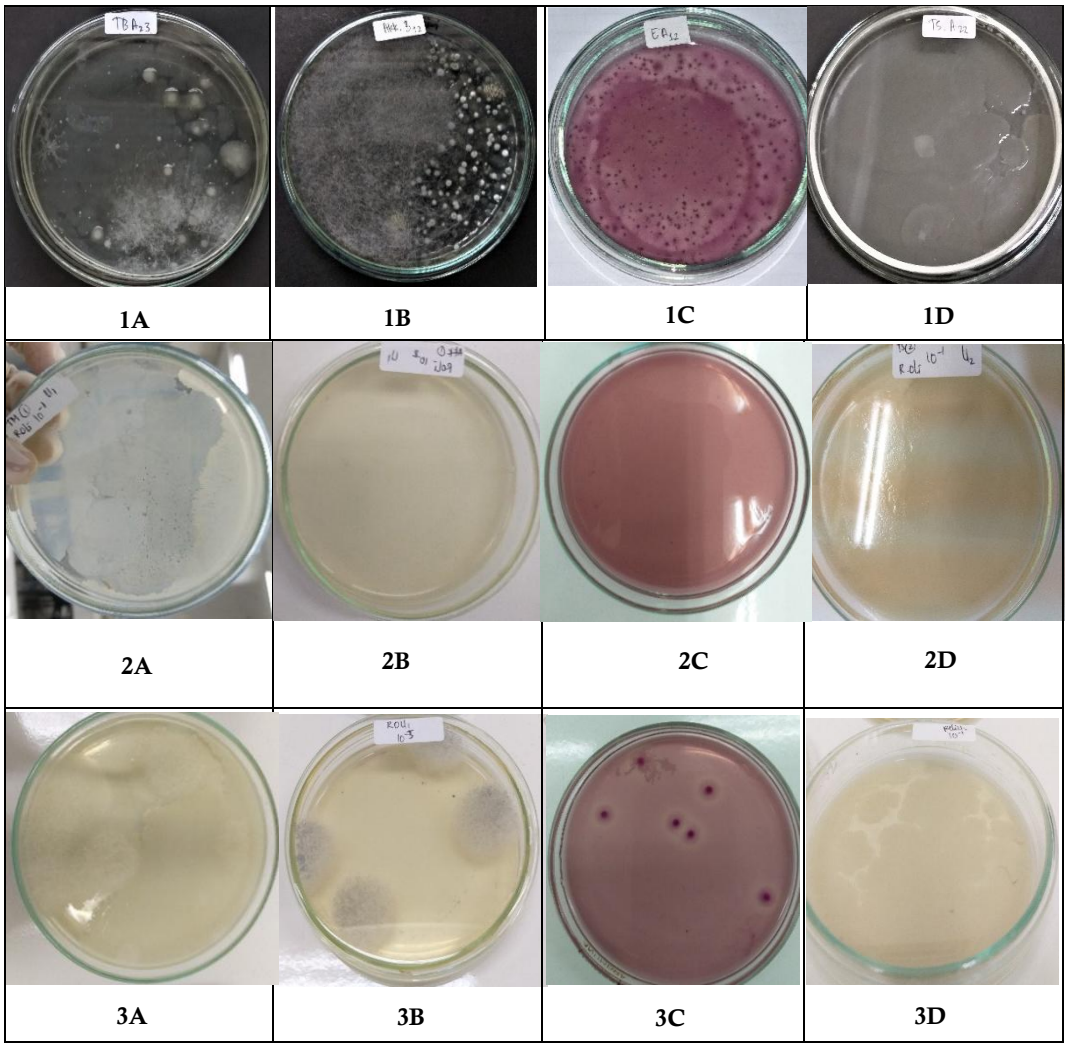


Figure 3. Macroscopic Observation of Microorganisms in Agar Media. Raw material black rice bran: total microbes (1A); total mould (1B); total *Enterobacteriaceae* (1C): total spores (1D). Pasteurized rice bran tempeh: total microbes (2A); total mould (2B); total *Enterobacteriaceae* (2C): total spores (2D). Unpasteurized rice bran tempeh: total microbes (3A); total mould (3B); total *Enterobacteriaceae* (3C): total spores (3D)

The total microbial study in this investigation aims to determine the number of microorganisms present in the sample, thereby assessing the product's cleanliness and safety. The results of this study show that several microbes continue to grow in the highest bran tempeh without pasteurization treatment (Table 1). However, it does not fully indicate the presence of contaminating bacteria. This is because the microbes that grow are Rhizopus mold, and lactic acid bacteria may also be present during the fermentation process. This assumption is supported by the results of studies showing that lactic acid bacteria, such as *L. plantarum*, can grow during the fermentation process under supportive environmental conditions, including an appropriate pH and nutrient availability. A study by Moon & Chang (2021) reported that the fermentation of bran with *L. plantarum* produced high cell concentrations and increased antimicrobial activity, indicating that LAB can live and develop actively in substrates such as bran.

Then, the identification of *Enterobacteriaceae* bacteria in this study is important because it is often used as an indicator of contamination from pathogenic intestinal bacteria. Therefore, the presence of *Enterobacteriaceae* in food products indicates poor sanitation (Jerśek, 2017). *Enterobacteriaceae* bacteria, as reported by Jerśek (2017), include *Salmonella, Escherichia coli, Proteus, Shigella, Yersinia, Serratia, Klebsiella,* and *Enterobacter*, which can cause disease. *Enterobacteriaceae* bacteria were detected in relatively large numbers in raw bran materials (Table 1), whereas in pasteurized bran tempeh, the numbers were relatively low. This is evidenced by the results of macroscopic observations (Figures 1C – 3C), where several *Enterobacteriaceae* colonies were seen growing on VRBGA selective media. In the raw materials of bran (1C) and unpasteurized bran tempeh (3C), *Enterobacteriaceae* colonies were identified by their distinctive characteristics, which included a red or purple color and the presence or absence of a precipitation zone around them. This characteristic is evident in the traits of *Enterobacteriaceae* colonies in VRBGA media, specifically their ability to ferment glucose and form acids that react with neutral red indicators, as well as their capacity to cause bile salt precipitation around the colonies (ISO 21528-2:2017).

Additionally, the presence of Enterobacteriaceae can also cause food safety. Several genera in this family, such as *Salmonella* and *E. coli*, have been reported to produce toxins that cause food poisoning (Alhadlaq et al., 2024; FDA, 2022). Thus, the success of the pasteurization process in reducing the number of Enterobacteriaceae demonstrates that this thermal treatment is crucial in microbiological control. The pasteurization process can be categorized as one of the critical control points (CCPs) in the HACCP (Hazard Analysis and Critical Control Points) system, which aims to control biological hazards. Thus, the pasteurization process is effective in reducing the number of Enterobacteriaceae, thereby increasing food safety).

CONCLUSION

The pasteurization process on fermented bran tempeh with *R. oligosporus* and *R. oryzae* cultures for 10 minutes effectively reduced the number of contaminating microorganisms, including total microbes, molds, *Enterobacteriaceae*, and spores. Quantitatively, in pasteurized R. oryzae cultured bran tempeh, the total number of microbes decreased to 1.45 log CFU/g compared to 3.30 log CFU/g without pasteurization. Meanwhile, in *R. oligosporus* culture, the total microbial value after

pasteurization was 2.46 log CFU/g, decreasing from 3.46 log CFU/g in samples without pasteurization. In contrast, in fermented bran tempeh with *R. oryzae* culture, pasteurization gave a greater quantitative reduction effect. It tended to be more consistent in all microbiological parameters, including total microbes (decreased from 3.46 to 1.45 log CFU/g), total molds (from 3.64 to <1 log CFU/g), total *Enterobacteriaceae* (<1 log CFU/g), and total spores (from 2.63 to <1 log CFU/g), although not all were statistically significant. This study was limited to a pasteurization duration of 10 minutes, and no specific identification of the microorganisms found was carried out, nor was the identification of the effect of pasteurized bran tempeh on the content of bioactive compounds. This finding scientifically verifies the selective effectiveness of pasteurization in reducing microbial hazards in fermented foods based on bran, and as a means of confirming its application in a home industry and Micro, Small and Medium Enterprises (MSMEs) to enhance product safety and shelf life.

REFERENCES

Alhadlaq, M. A., Aljurayyad, O. I., Almansour, A., Al-Akeel, S. I., Alzahrani, K. O., Alsalman, S. A., Yahya, R., Al-Hindi, R. R., Hakami, M. A., Alshahrani, S. D., Alhumeed, N. A., Al Moneea, A. M., Al-Seghayer, M. S., AlHarbi, A. L., Al-Reshoodi, F. M., & Alajel, S. (2024). Overview of pathogenic *Escherichia coli,* with a focus on Shiga toxin-producing serotypes, global outbreaks (1982-2024) and food safety criteria. *Gut pathogens*, *16*(1), 57. https://doi.org/10.1186/s13099-024-00641-9

Ardiani, I., Aýun, Q., & Nazua, K. S. (2024). Variations in Rhizopus Species That Play a Role in Making Tempeh in the Bekasi Area. *Biosel (Biology Science and Education): Jurnal Penelitian Science dan Pendidikan*, *13*(1), 10-18. https://doi.org/10.33477/bs.v13i1.6816

A'yun, Q., & Janah, L. U. (2022). Isolation of *R. Oligosporus* and *R. Stolonifer* in Three Types of Tempeh in Jatimakmur Village, Bekasi. *Konservasi Hayati, 18*(2), 44–50. https://doi.org/10.33369/hayati.v18i2.23801 **[*In Indonesian language*]**

[BAM] Bacteriological Analytical Manual. (2001a). *Aerobic Plate Count.* US FDA, Center for Food Safety and Applied Nutrition.

[BAM] Bacteriological Analytical Manual. (2001b). *Yeasts, Molds and Mycotoxins.* US FDA, Center for Food Safety and Applied Nutrition.

Budijanto, S., Nurtiana, W., Muniroh, A., Kurniati, Y., Nuraida, L., Priosoeryanto, B. P., Dewi, F. N. A., Ardiansyah A, Yuliana, N. D., Safrida, S, & Shirakawa, H. (2023). Dietary supplementation of black rice bran to colon carcinogen-induced mice: Examining the development of colorectal cancer by improving environmental colon conditions. *Heliyon*, *9*(8), e18528. https://doi.org/10.1016/j.heliyon.2023.e18528

Budijanto, S., Kurniati, Y., Nuraida, L., Dewi, F. N. A., Priosoeryanto, B. P., Yuliana, N. D., Ardiansyah, Saepuloh, U., Safrida, & Shirakawa, H. (2024). The inhibition of colon cancer development by black rice bran on BALB/C

Mice. *AIMS Agriculture and Food*, *9*(3), 789-800. https://doi.org/10.3934/agrfood.2024042

[ISO] International Organization for Standardization 21528-2. (2017). *Microbiology of The Food Chain-Horizontal Method for The Detection and Enumeration of Enterobactericeae. Colony-count technique*. Switzerland : International Standard.

Jeršek, B. (2017). *Microbiological Examination of Food*. Slovenia: Ljubljana University.

[FDA] (Food & Drug Administration U.S.). (2022). *Bad Bug Book: Handbook of Foodborne Pathogenic Microorganisms and Natural Toxins*. https://www.fda.gov/media/83271/download

Harrigan, W. F. (1998). *Laboratory Methods in Food Microbiology*. 3th ed. San Diego: Academic Pr.

Moon, S. H. & Chang, H.-C. (2021). Rice Bran Fermentation Using Lactiplantibacillus plantarum EM as a Starter and the Potential of the Fermented Rice Bran as a Functional Food. *Foods* (10), 978. https://doi.org/10.3390/foods10050978

Nurtiana, W. (2018). The potential of black rice bran to alter short-chain fatty acid concentrations and β-glucuronidase enzyme activity as an inhibitor of colon cancer development in vivo. Magister Thesis of Institut Pertanian Bogor. http://repository.ipb.ac.id/handle/123456789/92498 **[*In Indonesian language*]**

Purwijantiningsih, E., Dewanti-Hariyadi, R., Nurwitri, C. C., & Istiana, I. (2019). Inhibition of Aflatoxin Production from Aspergillus flavus by Moulds and Yeasts Isolated from Tempeh Yeast. *Biota: Jurnal Ilmiah Ilmu-Ilmu Hayati*, *10*(3), 146–153. https://doi.org/10.24002/biota.v10i3.2874 **[*In Indonesian language*]**

Rodhibilah, F. I., Rosalina, R., Akbar, F., Yuliana, T., Ningrum, R. S., & Safitri, R. (2023). Fabrication and Properties Evaluation of *R. oligosporus* Mycelium-Based Biofoam with Various Lignocellulosic Substrates. *Biosciences Research, 20*(2), 518-529.

Safrida., Budijanto, S., Nuraida, L., Priosoeryanto, B. P., Saepuloh, U., Marya, S. S., Ardiansyah, & Shirakawa, H. (2022). Fermented black rice bran extract inhibit colon cancer proliferation in WiDr cell lines. *Food Science and Technology*, *42*, e14422. https://doi.org/10.1590/fst.14422

Setlow, P. (2016). *Spore resistance properties. The bacterial spore: from molecules to systems.* Hoboken: John Wiley & Sons, Inc., Page: 201-215.

Tuncel, N. Y. (2023). Stabilization of rice bran: a review. *Foods*, *12*(9), 1924. https://doi.org/10.3390/foods12091924

Wen, J., Smelt, J. P., Vischer, N. O., de Vos, A. L., Setlow, P., & Brul, S. (2022). Heat activation and inactivation of bacterial spores: is there an overlap?. *Applied and Environmental Microbiology*, *88*(5), e02324-21. https://doi.org/10.1128/aem.02324-21

Yastrebov, K. (2025). Sterilization: The Science of Eliminating Microbial Contamination. *Journal of Medicine and Medical Sciences*, *16* (1), 1-3, https://doi.org/10.14303/2141-9477.2025.139

Zulfafamy, K. E., Ardiansyah, A., & Budijanto, S. (2018). Antioxidative properties and cytotoxic activity against colon cancer cell WiDr of Rhizopus oryzae and Rhizopus oligosporus-fermented black rice bran extract. *Current Research in Nutrition and Food Science*, *6*(1), 23-34. https://doi.org/10.12944/CRNFSJ.6.1.03

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