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Microbiological characteristics of tofu waste fermented with variations in microbial consortium and starter levels

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ABSTRACT

Feed as a source of livestock nutrients often experiences fluctuations in price and quality. Feed optimization can be achieved by seeking alternative, affordable feed sources such as processed tofu dregs to enhance its utility. The fermentation method using a consortium of microbes like fermented vegetable extract (FVE) 2019, tape yeast, and tempeh yeast is expected to work synergistically to improve the quality and utility of tofu dregs. This research aims to determine the effect of using variations of microbial consortia and different starter levels on the microbiological characteristics of tofu dregs. This study used a completely randomized design with a 3 × 7 factorial pattern and 3 replications. The first factor was the combination of microbial consortia, namely K0 (100% FVE-2019), K1 (50% FVE-2019+50% tape yeast), K2 (50% FVE-2019+50% tempeh yeast), K3 (50% FVE-2019+25% tape yeast+25% tempeh yeast), K4 (40% FVE-2019+30% tape yeast+30% tempeh yeast), K5 (100% tape yeast), K6 (100% tempeh yeast). The second factor was the starter levels: A1 (3%), A2 (4%), and A3 (5%). Data were analyzed using ANOVA test at 5% and Duncan's significant test at 5% level. The parameters observed in this study were microbiological performance and organoleptic. The conclusion of this study is FVE-2019 and tempeh yeast at a 5% level in fermentation was proven to improve the microbiological characteristics in terms of high LAB counts and the suitability of tofu waste, making it a potential source of functional feed ingredients.

Introduction

Feed plays an important role as a source of nutrients for the growth and livelihood of livestock. Farmers often face challenges in meeting the need for high-quality feed at affordable prices. The cost of feed, which accounts for up to 70% of total livestock production expenses, drives farmers to improve their feed efficiency (Sugiarti *et al*., 2024). The availability of economical, high-quality feed is one of the challenges to achieving optimal productivity (Amole *et al*., 2022). Fluctuations in raw material prices, uncertain availability, and competition for land with food crops all contribute to the challenges faced by the livestock industry. These conditions force farmers to innovate and find strategies to continue obtaining low-cost feed with optimal quality.

Feed optimization efforts can be carried out by seeking alternative feeds. This optimization can help reduce production costs while also increasing feed formulation efficiency. Alternative feeds commonly used by farmers include agricultural industry waste (Quintero-Herrera *et al*., 2023). Large amounts of industrial waste have the potential to be used as local feed ingredients due to their abundant availability (Yafetto *et al*., 2023). Some agricultural industry waste still contains fairly high levels of nutrients. An example of economical agricultural industry waste that can be utilized by farmers is tofu dregs (Kusumaningtyas *et al*., 2020).

Tofu dregs is the fine residue left over from the soybean-based tofu production industry. Based on 2015 BPS data, there were 75 tofu factories in Semarang, 1 tofu factory can produce up to 83 sacks of tofu dregs/day (Sugianti, 2018), so that tofu dregs are abundant and easy to obtain at affordable prices. Tofu dregs contain 20.65% crude protein, 1.72% crude fat, 18.44% crude fiber, and 3.14% ash (Mulyasari *et al*., 2022). Abundant availability also supports the potential of tofu dregs as a local feed ingredient. However, the utility of tofu dregs given directly to livestock is lower compared to processed tofu dregs. This occurs because tofu dregs have a high crude fiber content, which can increase N excretion and inhibit digestibility (Zhang *et al*., 2024). In addition, tofu dregs are susceptible to contamination by bacteria such as coliform. The processing method that can be used to increase the utility of tofu dregs is fermentation using microorganisms. Microorganisms will use tofu dregs as a substrate because they contain soybean oligosaccharides (SBOS) (Du *et al*., 2023).

Some sources of microorganisms used for fermentation are fermented vegetable extract (FVE) 2019 (Sulistiyanto *et al*., 2024), tape yeast, and tempeh yeast. These starters are known to contain various types of microorganisms such as lactic acid bacteria (Lactobacillus spp.) in FVE-2019 (Sulistiyanto *et al*., 2019), *Saccharomyces cerevisiae* in tape yeast (Gunam *et al*., 2021), and *Rhizopus* sp. in tempeh yeast (Tamang *et al*., 2022). The combination of several microorganisms can form a microbial consortium that has the potential to serve as a fermentation starter (Fabricio *et al*., 2023). A microbial consortium is a mixture of several types of microorganisms that work synergistically (Maheshwari *et al*., 2023). Fermentation using a consortium of microbes has the potential to improve the quality and utility of tofu pulp by increasing its nutritional value, digestibility, as well as the availability of protein and bioactive compounds.

In addition to improving the quality of tofu pulp, these microorganisms are also capable of producing single cell protein (SCP) (Sharif *et al*., 2021). SCP is biomass of microorganisms that have died and dried, which grow on different carbon and energy sources (Jach *et al*., 2022). The activity of lactic acid bacteria, yeast, and certain molds within a consortium can increase the probiotic population while simultaneously inhibiting the growth of pathogens, thereby enhancing the microbiological safety of the final product. This condition makes microbial consortia a potential alternative to antibiotics in modern livestock systems.

This study aimed to examine the effects of fermenting tofu dregs using different microbial consortium variations and starter levels on their microbiological characteristics. The goal is to provide insights into how these factors influence the quality of tofu dregs.

Materials and methods

Research materials

The materials used in this research include tofu dregs, molasses, FVE-2019, Raprima brand tempeh yeast, and NKL brand tape yeast. The

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equipment used includes sterile jars, a digital scale, an analytical balance, a measuring cup, petri dishes, and a stirring spatula.

Experimental design

The research was carried out experimentally using a Complete Random Design (CRD) factorial pattern of 3 x 7 treatments, and 3 replications. The first factor is the combination of microbial consortia, which are K0 (100% FVE-2019), K1 (50% FVE-2019+50% tape yeast), K2 (50% FVE-2019+50% tempeh yeast), K3 (50% FVE-2019+25% tape yeast+25% tempeh yeast), K4 (40% FVE-2019+30% tape yeast+30% tempeh yeast), K5 (100% tape yeast), and K6 (100% tempeh yeast). The second factor is the level of starter, namely A1 (3%), A2 (4%), and A3 (5%). The combination of research treatments is as follows:

K0A1= Fermented tofu dregs with 3% (100% FVE-2019)
K0A2= Fermented tofu dregs with 4% (100% FVE-2019)
K0A3= Fermented tofu dregs with 5% (100% FVE-2019)
K1A1= Fermented tofu dregs with 3% (50% FVE-2019+50% tape yeast)
K1A2= Fermented tofu dregs with 4% (50% FVE-2019+50% tape yeast)
K1A3= Fermented tofu dregs with 5% (50% FVE-2019+50% tape yeast)
K2A1= Fermented tofu dregs with 3% (50% FVE-2019+50% tempeh yeast)
K2A2= Fermented tofu dregs with 4% (50% FVE-2019+50% tempeh yeast)
K2A3= Fermented tofu dregs with 5% (50% FVE-2019+50% tempeh yeast)
K3A1= Fermented tofu dregs with 3% (50%FVE-2019+25% tape yeast+25% tempeh yeast)
K3A2= Fermented tofu dregs with 4% (50% FVE-2019+25% tape yeast+25% tempeh yeast)
K3A3= Fermented tofu dregs with 5% (50% FVE-2019+25% tape yeast+25% tempeh yeast)
K4A1= Fermented tofu dregs with 3% (40% FVE-2019+30% tape yeast+30% tempeh yeast)
K4A2= Fermented tofu dregs with 4% (40% FVE-2019+30% tape yeast+30% tempeh yeast)
K4A3= Fermented tofu dregs with 5% (40% FVE-2019+30% tape yeast+30% tempeh yeast)
K5A1= Fermented tofu dregs with 3% (100% tape yeast)
K5A2= Fermented tofu dregs with 4% (100% tape yeast)
K5A3= Fermented tofu dregs with 5% (100% tape yeast)
K6A1= Fermented tofu dregs with 3% (100% tempeh yeast)
K6A2= Fermented tofu dregs with 4% (100% tempeh yeast)
K6A3= Fermented tofu dregs with 5% (100% tempeh yeast)

Research procedure

The research stage began by creating combinations of microbial consortia according to the treatments. The next step involved weighing the tofu dregs based on the requirements of each treatment. The tofu dregs was mixed with 3% molasses as an additional energy source for the growth of microorganisms. The prepared starter combinations were then weighed according to the treatment levels (3%, 4%, and 5%) based on the weight of the tofu dregs. The evenly mixed tofu dregs and molasses mixture was then added with the starter and anaerobic fermentation is carried out for 5-7 days. The tofu dregs are fermented anaerobically in sterile perforated jars and stored in a dark place.

The observed parameters consisted of microbiological characteristics, color changes, pH, and mold appearance. Microbiological data were obtained from the total count of bacteria including lactic acid bacteria (LAB), total molds, and Coliform using the Total Plate Count (TPC) method. Observations of color changes, pH, and mold appearance were conducted at hour 160, and subsequently tabulated based on harvest time.

The Coliform test begins by taking a 5-gram sample and performing a 1:10 and 1:100 dilution. The dilution is done in duplicate by adding TSB/SCDB up to 50 mL and homogenizing the mixture. The sample is then incubated at a temperature of 30-35°C for 18 - 24 hours. A sterile membrane filter with a diameter of 47 mm (other diameters can also be used) and a pore size of 0.45 µm is used to filter the sample, which is then rinsed with 100 mL of MacConkey Broth (MCB) medium. The membrane filter is transferred onto MacConkey Agar (MCA) or Eosin Methylen Blue (EMB) media and incubated in an incubator at 30-35°C for 18-72 hours. The number of colonies that grow on the media is counted using the Total Plate Count (TPC) method.

LAB and total mold testing are conducted under Laminar Air Flow. A total of 2.0 mL/gram of the sample is taken and, if necessary, serially diluted. Then, 0.1 mL of the suspension is inoculated onto a petri dish containing sterile MRSA or SDA media, spread evenly, and incubated in duplicate. Incubation is carried out at 30–35°C for 3–5 days (MRSA) and 20–25°C for 5–7 days (SDA). After that, the colonies are observed and counted. If there are no colonies at the initial dilution (1:10), the result is stated as "not less than 10 microbes per gram/mL." The number of colonies growing on the media is counted according to the Total Plate Count (TPC) method.

Data analysis

Data analysis is carried out using a descriptive method. The data of the research results are compiled in the form of a table and then interpreted according to the results of existing observations. Microbiologis, color, pH, and fungi data were analyzed using a variety of analyses with a significance level of 5% to determine the effect of treatment. If there is an effect of treatment, a further test is carried out, namely the Duncan Double Area Test to find out the difference between treatments.

Results

This study examines the microbiological aspects of tofu dregs fermented using various microorganism consortia and different starter levels after incubation for 160 hours. These aspects are supported by observational data, including the appearance of mold, color changes, and pH. The research results are presented in Tables 1 and 2.

Discussion

The results of the study showed an interaction ($P<0.05$) between the microbial consortium and the level of starter on the total lactic acid bacteria (LAB), total fungi, and total Coliform. The total number of LAB in the fermented tofu dregs ranged from 7.28 to 7.85 Log CFU/g. (Supriya *et al*., 2023) states that fermented feed with a LAB count of ≥ 10⁶ CFU/g (6 Log CFU/g) able to provide beneficial effects, such as inhibiting pathogens and improving fermentation quality. Fermented feed with a LAB content of 10^5 CFU/g (5 Log CFU/g) given to poultry can improve their growth performance (Wang *et al*., 2023). K2A3 treatment (7.85 Log CFU/g) had the highest total LAB compared to other treatments. This indicates that the microbial consortium derived from FVE-2019 and 5% tempeh yeast in tofu dregs was able to increase the number of LAB. The FVE-2019 starter contains various types of microorganisms such as lactic acid bacteria (Lactobacillus spp.) (Sulistiyanto *et al*., 2019) that it supports the growth of lactic acid bacteria in the tofu dregs fermentation process. The activity of tempeh yeast can increase the number of LAB by providing a carbon source as a nutrient (Rizal *et al*., 2021). Tempeh yeast can convert the components of tofu waste into forms that are easier to digest and create a favorable environment for the growth of lactic acid bacteria (LAB). The combination of consortia with different starter levels is effective in increasing the population of LAB during the fermentation process.

The research results show an interaction ($P<0.05$) between the microbial consortia and starter levels on the total fungi. The findings indicate

Table 1. TPC BAL, total fungi, and coliform of tofu waste fermented with variations of microbial consortium and starter levels after 160 hours of storage.

Parameter	Consortium (K)	Starter level (A)		
		A1	A2	A3
LAB (log CFU/g)	K0	7.68^{cdefg+}0.04	7.61^{fghi}±0.02	7.62^{efgh}±0.01
	K1	7.52^{ij}±0.03	7.70^{cde}±0.05	7.65^{defgh}±0.00
	K2	7.84^{ab}±0.03	7.76^{bc}±0.02	7.85^{a}±0.01
	K3	7.41^{k}±0.09	7.72^{cd}±0.09	7.58^{hi}±0.03
	K4	7.59^{ghi}±0.00	7.60^{fghi}±0.01	7.66^{defgh}±0.00
	K5	7.49^{j}±0.11	7.28^{l}±0.07	7.64^{defgh}±0.03
	K6	7.38^{k}±0.07	7.69^{cdef}±0.02	7.59^{ghi}±0.07
Total Fungi (log CFU/g)	K0	6.03^{g}±0.06	6.72^{cd}±0.00	6.84^{b}±0.02
	K1	6.12^{f}±0.05	5.76^{i}±0.08	4.65^{l}±0.01
	K2	6.99^{a}±0.01	6.26^{e}±0.00	5.80^{i}±0.03
	K3	6.77^{c}±0.03	6.26^{e}±0.05	6.19^{e}±0.05
	K4	5.11^{k}±0.01	6.07^{fg}±0.01	5.67^{j}±0.02
	K5	6.20^{e}±0.04	6.76^{cd}±0.02	5.95^{h}±0.04
	K6	6.69^{d}±0.07	6.75^{cd}±0.03	6.70^{d}±0.07
Coliform (log CFU/g)	K0	4.03^{de}±0.09	3.70^{g}±0.31	3.87^{f}±0.01
	K1	4.06^{cd}±0.03	3.90^{ef}±0.13	4.03^{de}±0.03
	K2	4.07^{cd}±0.02	3.80^{fg}±0.05	4.05^{cd}±0.01
	K3	4.29^{ab}±0.01	4.12^{cd}±0.06	3.87^{f}±0.03
	K4	4.20^{bc}±0.00	4.18^{bcd}±0.00	4.20^{bc}±0.00
	K5	4.20^{bc}±0.00	4.15^{bcd}±0.03	3.68^{g}±0.01
	K6	4.13^{cd}±0.01	4.35^{a}±0.02	4.17^{bcd}±0.02

Different superscripts in the rows and columns of the interaction indicate significant differences (P<0,05). K0 = 100% FVE-2019; K1 = 50% FVE-2019 + 50% tape yeast; K2 = 50% FVE-2019 + 50% tempeh yeast; K3 = 50% FVE-2019 + 25% tape yeast + 25% tempeh yeast; K4 = 40% FVE-2019 + 30% tape yeast + 30% tempeh yeast; K5 = 100% tape yeast; K6 = 100% tempeh yeast; A1 = Starter level 3%; A2 = Starter level 4%; A3 = Starter level 5%.

Table 2. Color changes, pH value, and mold appearance in tofu dregs fermented with variations in microbial consortium and starter levels during 160 hours of storage.

Parameter	Consortium (K)	Starter level (A)		
		A1	A2	A3
Color Changes	K0	1.00^{c}±0	1.00^{c}±0	1.00^{c}±0
	K1	3.00^{a}±0	1.00^{c}±0	1.67^{b}±1.15
	K2	1.00^{c}±0	1.00^{c}±0	1.00^{c}±0
	K3	1.00^{c}±0	1.00^{c}±0	3.00^{a}±0
	K4	3.00^{a}±0	1.00^{c}±0	1.00^{c}±0
	K5	3.00^{a}±0	3.00^{a}±0	3.00^{a}±0
	K6	2.00^{b}±0	2.00^{b}±0	2.00^{b}±0
pH Value	K0	5.33^{b}±0.58	5.33^{b}±0.58	5.00^{b}±0
	K1	5.00^{b}±0	5.00^{a}±0	5.00^{b}±0
	K2	5.33^{b}±0.58	5.33^{b}±0.58	5.00^{b}±0
	K3	5.00^{b}±0	5.00^{a}±0	5.00^{b}±0
	K4	5.33^{b}±0.58	5.33^{b}±0.58	5.00^{b}±0
	K5	5.33^{b}±0.58	5.00^{b}±0	4.00^{c}±0
	K6	6.00^{a}±0	6.00^{a}±0	6.00^{a}±0
Mold Appearance	K0	Negative	Negative	Negative
	K1	Positive	Negative	Positive
	K2	Negative	Negative	Negative
	K3	Negative	Negative	Positive
	K4	Positive	Negative	Negative
	K5	Positive	Positive	Negative
	K6	Positive	Negative	Positive

Different superscripts in the rows and columns of the interaction indicate significant differences (P<0,05). K0 = 100% FVE-2019; K1 = 50% FVE-2019 + 50% tape yeast; K2 = 50% FVE-2019 + 50% tempeh yeast; K3 = 50% FVE-2019 + 25% tape yeast + 25% tempeh yeast; K4 = 40% FVE-2019 + 30% tape yeast + 30% tempeh yeast; K5 = 100% tape yeast; K6 = 100% tempeh yeast; A1 = Starter level 3%; A2 = Starter level 4%; A3 = Starter level 5%. Color score 1=brown; score 2=light brown; score 3=dark brown

that the total fungi in fermented tofu waste ranges from 4.65-6.99 Log CFU/g. This value is still within the tolerance limits for probiotic microorganisms to work optimally, on 106 CFU/g (Nurdianto *et al*., 2015). Treatment K2A1 shows the highest total fungal yield compared to the other treatments. The microbial consortium using FVE, tape yeast, and tempeh yeast produced fungi *Saccharomyces cerevisiae* (Gunam *et al*., 2021), and *Rhizopus* sp. (Tamang *et al*., 2022) as a probiotic in animal feed (Cuenca *et al*., 2022). *Rhizopus* spp. can improve gut health and nutrient absorption in livestock, potentially leading to better growth performance and feed efficiency (Hamza & Gunyar, 2022).

The research results showed that there was a significant interaction (P<0.05) between the microbial consortium and starter levels on the total Coliform. Total Coliform falls within the range of 3.68-4.35 Log CFU/g, the lower the total Coliform value, the better the microbiological quality. Coliform bacteria serve as an indicator of pathogens in animals and humans, as the number of colonies is positively correlated with the presence of pathogenic bacteria (Purba *et al*., 2022). The low total Coliform count in tofu dregs fermented with various microbial consortia and starter levels is due to the high levels of lactic acid bacteria and the influence of *Saccharomyces cerevisiae*, which produces yeast. An important function of LAB (lactic acid bacteria) is balancing the gut microbiota (Dahiya & Nigam, 2022). LAB can increase the population of beneficial bacteria like *Bifidobacterium* spp. while reducing harmful bacteria such as *Enterobacteria* spp. and *Escherichia coli* (Sirisopapong *et al*., 2023). Saccharomyces has antibiotic properties because it produces ethanol, which can inhibit a pathogen.

The K2A3 treatment with the highest amount of LAB had the lowest color change score, which was 1 (brown). This score resulted from the enzymatic activity of lactic acid bacteria. The application of LAB in the fermentation process can increase color brightness due to the degradation of complex pigments (Zhu *et al*., 2022). The application of LAB in feed fermentation can also lower the pH, making it more acidic. A more acidic environment can inhibit the growth of pathogens and reduce the risk of contamination by other microorganisms (Barron *et al*., 2024). LAB produces lactic acid and antimicrobial compounds that are not conducive to the growth of microorganisms, including fungi. This can be seen from the observation results of treatment K2A3 at hour 160, where no fungal growth was observed. The growth of fungi, including *Rhizopus* sp., tends to be inhibited when LAB lowers the pH. <5 (Fatima *et al*., 2024).

The K2A1 treatment with the highest total fungi had the lowest color change score, which was 1 (brown). This score resulted from the activity of fungal secondary metabolites. The application of yeast in the fermentation process caused the feed color to become uneven, due to both pigment degradation and the production of secondary metabolite pigments (Echeverrigaray *et al*., 2020). The application of tempeh yeast in feed fermentation tends to lower the pH from alkaline or neutral to acidic (Yoshida *et al*., 2022). Dynamic changes in pH during fermentation can affect the growth, morphology, and physiology of the microorganisms involved in fermentation, and may even trigger the growth of spoilage microbes. Based on this, the fermentation process using tempeh yeast can be combined with a starter source of LAB to maintain pH stability (Teoh *et al*., 2024). This condition can be seen from the observation results of the K2A1 treatment at the 160th hour; this treatment did not show any fungal growth due to the acidic conditions produced by LAB.

K5A3 treatment with the lowest total Coliform had the highest color change score, which was 3 (dark brown). This result occurred due to the activity of secondary metabolites produced by the fungus. The application of tape yeast in the fermentation process makes the feed color unstable. *Saccharomyces cerevisiae* (tape yeast) carries out alcoholic fermentation that produces volatile compounds and acids, such as ethanol

and organic acids. These compounds can cause color fading, yellowing, or other color changes due to secondary chemical reactions (Morata *et al*., 2019). This instability also occurred in the pH value after fermentation. Nevertheless, volatile compounds in the form of ethanol, a secondary metabolite produced by *Saccharomyces cerevisiae*, were able to suppress the growth of pathogens, as evidenced by the absence of mold on K5A3. Ethanol can damage the cell membranes of pathogens and cause protein denaturation (Yoshida *et al*., 2022).

Based on these results, it can be seen that LAB, *Saccharomyces cerevisiae*, and *Rhizopus* sp. have potential as probiotic sources for fermenting tofu dregs. Tofu dregs can serve as a prebiotic source because they contain insoluble fiber and complex carbohydrates, such as raffinose and stachyose. Fermentation of tofu dregs by these three microorganisms can increase nutrient availability and enrich the content of bioactive metabolites such as digestive enzymes, organic acids, and antimicrobial compounds. (Vuscan *et al*., 2024) The cell wall components of *Saccharomyces cerevisiae* can enhance immune responses, while *Rhizopus* sp. is capable of producing protease and phytase enzymes (Mohamed Abdullah Maitig *et al*., 2018) which can increase the digestibility of amino acids (Sureshkumar *et al*., 2023). The use of tofu dregs fermented with FVE, tape yeast, and tempeh yeast has the potential to serve as functional feed, due to the bioactive metabolite content that works synergistically to improve digestive tract health and disease resistance.

Conclusion

Based on the research results, it can be concluded that the use of FVE and tempeh yeast at a 5% level in fermentation has been proven to improve microbiological characteristics in terms of a high number of lactic acid bacteria and the viability of tofu dregs, thus making it a potential source of functional feed ingredients.

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Conflict of interest

The authors have no conflict of interest to declare.

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