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Interaction effect of inoculum type and dosage in the fermentation process on the quality of soy milk waste (SMW) for animal feed

Pengaruh interaksi antara jenis dan dosis inokulum dalam proses fermentasi terhadap kualitas soy milk waste (SMW) untuk pakan ternak

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A B S T R A C T

The aim of this research is to determine the effect of the interaction between the type and dose of inoculum in fermentation to improve the quality of soy milk waste (SMW) for animal feed. This study used a completely randomized 2 x 5 factorial design. The first factor was the type of inoculum (*Saccharomyces cerevisae* and *Rhizopus* sp.) and the second factor was the inoculum dose (0, 5, 10, 15 and 20%). Each treatment was repeated 4 times. The results of statistical analysis showed that the type factor, dosage factor of the inoculum, and the interaction between the two factors had a very significant ($p<0.01$) effect on the quality of color, aroma, sugar concentration and alcohol concentration of SMW. In this study, the color score from SMW was found to be between 3.00-5.00; texture scores range from 1.00 to 4.50; moisture 13.23-15.03%; sugar concentration is 0.00-5.63%, and alcohol concentration ranges from 0.00-1.43%. This research can be concluded that the interaction between the type and dose of inoculum in fermentation can improve the quality of soy milk waste (SMW). The best quality was obtained when using *Saccharomyces cerevisae* inoculum at a dose of 15%.

A B S T R A K

Tujuan dari penelitian ini untuk mengetahui pengaruh interaksi jenis dan dosis inokulum dalam fermentasi untuk memperbaiki kualitas soy milk waste (SMW) untuk pakan ternak. Penelitian ini menggunakan rancangan acak lengkap faktorial 2 x 5. Faktor pertama adalah jenis inokulum (Saccharomyces cerevisae dan Rhizopus sp) dan faktor kedua adalah dosis inokulum (0, 5, 10, 15, dan 20%). Setiap perlakuan diulang sebanyak 4 kali. Hasil analisis statistik menunjukkan bahwa faktor jenis, faktor dosis dari inokulum, dan interaksi antara kedua faktor sangat nyata (p<0,01) berpengaruh terhadap kualitas warna, tekstur, konsentrasi gula dan konsentrasi alkohol SMW. Pada penelitian ini didapatkan skor warna dari SMW berkisar antara 3,00-5,00; skor tekstur berkisar 1,00-4,50; kadar air 13,23-15,03%; konsentrasi gula 0,00-5,63%, dan konsentrasi alkohol berkisar 0,00-1,43%. Penelitian ini dapat disimpulkan bahwa interaksi antara jenis dan dosis inokulum dalam fermentasi dapat meningkatkan kualitas soy milk waste (SMW). Kualitas terbaik didapatkan pada penggunaan inokulum Saccharomyces cerevisae dengan dosis sebesar 15%.

Kata kunci:
Fermentasi
Rhizopus sp
Saccharomyces cerevisae
Soy milk waste

INTRODUCTION

Indonesia is one of the countries in Southeast Asia that has quite serious challenges in developing livestock farming (Edi & Haryuni, 2023). The poultry industry is one of the industries that absorbs a lot of labor. This industry is reported to be able to absorb around 12 million workers (Haryuni, Hartutik, Widodo, & Wahjuningsih, 2022). The poultry industry has shown rapid development in the last few decades. This is partly due to changes in the management system in modern poultry farms, where the management system applies data-based technology to manage the livestock business (Akbar, Haryuni, & Lestariningsih, 2024). Poultry livestock productivity is one of the parameters used to evaluate the level of success in livestock farming (Almi, Lestariningsih, & Haryuni, 2024). Factors that affect the level of productivity in livestock include internal factors (genetics) and external factors that include environmental temperature, poultry maintenance systems and feed. Each livestock has different nutritional requirements, this is due to differences in livestock types, age, body weight, environmental conditions and physiological conditions of livestock (Rohman, Lestariningsih, & Haryuni, 2024). The assessment of the quality of animal feed is based on the balance of the nutritional content in it with the appropriate amount of animal nutritional requirements. The feed consumed by livestock is used to supply energy and other nutrients used by livestock for maintenance (basal metabolism for basic life such as breathing, regulating body temperature, carrying out metabolic activities); production; and reproduction (Aldila, Haryuni, & Alam, 2023). Therefore, nutritional balance is an important factor that must be considered in formulating animal feed (Rizqita, Haryuni, & Lestariningsih, 2023). Animal feed is a mixture of several feed ingredients that have been calculated and adjusted for nutritional quality according to the livestock's nutritional requirements. While feed ingredients are all kinds of organic and inorganic materials that can be consumed by livestock to meet their nutritional requirements and are not harmful to the health and survival of livestock (Adi & Haryuni, 2024). In Indonesia, the problem related to feed that often occurs in livestock farming communities is the decreasing stock of plant-based feed ingredients such as corn. The decreasing stock of corn for feed is a serious problem because corn is the main component in animal feed that cannot be replaced in large quantities with other feed ingredients. The use of corn in poultry feed formulations reaches approximately 50% of the total feed ingredients (Nahroni, Haryuni, & Alam, 2023). Corn kernels have various colors, ranging from white, yellow, red, orange, purple, to black. This shows the richness of anthocyanin pigment compounds (anthocyanidin, aglycone, glucoside), carotenoids and others. Yellow corn is a type of corn that is generally used by livestock farmers in feed formulations (Bidura, 2016). Discussion about feed is often a trending topic because poultry feed costs are the largest component in production costs in the poultry business where the cost of purchasing feed reaches 94.03% of the total production costs. This certainly makes poultry farmers compete to get the best quality feed and cheap prices, where one alternative is through the use of local feed ingredients (Nasrullah, Lestariningsih, & Haryuni, 2022).

Local feed ingredients can be obtained from agricultural and livestock industry waste. Soy milk waste (SMW) is one of the industrial wastes obtained from the soy milk processing industry (Haryuni & Khopsoh, 2024). SMW has a high nutritional content, a fragile texture that makes it easy to digest when in the digestive tract. The high content of lactic acid bacteria (LAB) in SMW also makes SMW a source of probiotic feed. In addition, SMW also has a large amount of isoflavone content that can be used as a source of antioxidants to ward off excess free radicals in the body of livestock, thereby improving livestock health (Haryuni, Tribudi, Hasanah, & Prastya, 2024). SMW contains a number of monosaccharides and oligosaccharides consisting of glucose (0.20%), fructose (0.10%), galactose (0.20%), arabinose (1.00%), sucrose (0.60%), and stachyose + raffinose (1.40%) with 0.50% starch. SMW also contains fatty acids such as linoleic acid (54.10%), oleic acid (20.40%), palmitic acid (12.30%), linolenic acid (8.80%), stearic acid (4.70%) and other nutritional components of soy products consisting of isoflavones, phytosterols, lignans, phytates, coumestans, and saponins (Ciptaan, Mirnawati, & Djulardi, 2021). The mineral content of SMW is also quite high. The minerals in SMW include potassium (286 mg/100g), zinc (3.14 mg/100g), copper (0.77

mg/100g), Fe (4.45 mg/100g), potassium (9.36 mg/g), and magnesium (0.026 mg/g) (Anggraeni, Husmaini, Sabrina, Zulkarnain, & Rossi, 2020). One of the problems of using SMW as animal feed is the high crude fiber content. The crude fiber content in SMW ranges from 6.06-18.15% (Haryuni et al., 2024). The crude fiber contained in SMW can be reduced by fermentation technology. Fermentation technology has long been known by the people of Indonesia and has been applied widely in society. This technology is also known as nanotechnology, which is a technology that applies the principle of metabolism with the help of microorganisms. The food industry in Indonesia that has long utilized fermentation technology includes tempeh, oncom, soy sauce, tape and so on (Hasanah, Pradana, Kustiawan, Nurkholis, & Haryuni, 2022). Fermentation is a biochemical process that involves microorganisms to produce energy and is generally carried out anaerobically (Haryuni et al., 2024). One type of microorganism that can produce amylase enzyme is *Saccharomyces cerevisae*. Although SMW contains high amount of crude fiber where crude fiber is complex carbohydrate, it should be remembered that SMW is solid waste produced from making soy milk where the main ingredient is soybeans. Soybeans are a source of protein so the fermentation process also requires microorganisms that are able to produce protease enzymes. One of the microorganisms that can produce protease enzymes is *Rhizopus* sp. (Hasanah, Wahyono, Subagja, & Haryuni, 2024). Considering the great potential of SMW to be used as animal feed and also the limiting factors of its use, a study is needed to determine the effect of the interaction between the type and dose of inoculum in fermentation to improve the quality of soy milk waste (SMW) for animal feed.

MATERIALS AND METHODS

This study used a completely randomized design (CRD) factorial with a 2 x 5 pattern. The first factor was the type of inoculum (S), namely 2 types of inoculum (*Saccharomyces cerevisae* and *Rhizopus sp*). The second factor was the dose of inoculum (D), namely 0, 5, 10, 15 and 20%. Each treatment in this study was repeated 4 times.

Prosedur Fermentasi

The fermentation process of soy milk waste (SMW) in this study used 2 types of inoculum, namely *Saccharomyces cerevisiae* and *Rhizopus* sp. The SMW to be fermented was given inoculum according to the treatment, both the type and dose of the inoculum. The addition of inoculum to SMW before the fermentation process was carried out in stages by placing SMW in a tray on the bottom layer, then the next layer was inoculum and then SMW was given again on the next layer and after that it was stirred until homogeneous and transferred to a jar and tightly closed. This inoculum was given according to the type of treatment and after that it was incubated for 72 hours at room temperature (Ridwan & Haryuni, 2024). After the fermentation process is complete, the SMW quality analysis is carried out by checking and measuring the quality of the fermented SMW.

Observed Parameters

The parameters observed included color, texture, moisture, sugar concentration, and alcohol concentration. Measurements of the parameters observed in this study were carried out using several measurement methods. The color and texture of SMW were measured through organoleptic observations with reference to the assessment scores presented in Table 1. The moisture of SMW was measured using a digital grain moisture meter by inserting the tip of the tool into the SMW and the numbers displayed on the screen of this tool were recorded as the amount of moisture of the SMW. The sugar concentration and alcohol concentration were measured using a refractometer.

Statistical Analysis

The data obtained were recorded, tabulated and then analyzed statistically using ANOVA with a completely randomized design (CRD) factorial

Table 1. Physical quality assessment scores of fermented SMW

Score	Assessment Parameters	
	Color	Texture
1	Black	Hard texture
2	Grey	Slightly firm and not lumpy
3	Dark brown	Crumb
4	Brown	A bit wet and lumpy
5	Light brown	Wet

(Ridwan & Haryuni, 2024)

with a 2x5 treatment. Statistical analysis was continued with the Duncan test if the results obtained provided a significant or very significant difference in effect (Susanti, 2015).

$$Yij = \mu + \alpha i + \beta j + (\alpha\beta)ij + \varepsilon ij$$

Yij : Observation response variables
μ : Observation result value
αi : Effect of inoculum type on level i
εij : Effect of experimental error
I : Treatment
J : Repetitions
(αβ)ij : Interaction between A and B at factor A level i, factor B level j
βj : Effect of inoculum dose on j level

RESULTS AND DISCUSSION

The quality of soy milk waste (SMW) which includes the quality of color, texture, water content, sugar concentration and alcohol concentration of the fermentation results is presented in Table 2.

Color

Color is a very important indicator for determining the quality of feed ingredients (Hasanah, Haryuni, & Wahyono, 2024). The results of statistical analysis showed that the factors of inoculum type, inoculum dose and interaction between the two factors (inoculum type and dose) had a significant effect (p<0.01) on the color quality of fermented SMW. The SMW color scores produced in this study ranged from 3.00-5.00. The best SMW color with a score of 5.00 was obtained using *Rhizopus* sp. inoculum with a dose of 15%. The results obtained in this study were higher than the study by Hasanah, Haryuni, et al. (2024) which obtained a color score of SMW fermented using EM4 of 2.75-3.75. Fermentation is a process that utilizes yeast to synthesize enzymes and uses these enzymes to convert substrates which are complex molecules into products with simpler molecules. The fermentation process occurs through the activity of microorganisms selected for fermentation. Microorganisms commonly

Table 2. Quality of fermented soy milk waste (SMW)

Treatment	Variables				
	Color	Texture	Moisture (%)	Sugar Concentration (%)	Alcohol Concentration (%)
Effect of inoculum type factor					
S1	3.45 ± 2.40^{a}	2.95 ± 4.58^{b}	14.25 ± 2.41	3.35 ± 7.79^{b}	0.78 ± 2.00^{b}
S2	4.40 ± 2.87^{b}	2.70 ± 3.43^{a}	14.46 ± 2.67	0.74 ± 2.34^{a}	0.00 ± 0.00^{a}
Effect of inoculum dose factor					
D0	3.00 ± 0.00^{a}	1.00 ± 0.00^{a}	13.28 ± 0.02^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
D1	3.75 ± 0.37^{b}	2.88 ± 0.06^{a}	$14.45\pm0,29^{ab}$	2.31 ± 0.28^{b}	0.30 ± 0.15^{a}
D2	3.88 ± 0.43^{b}	3.00 ± 0.00^{a}	14.65 ± 0.15^{b}	1.90 ± 0.74^{b}	0.33 ± 0.16^{a}
D3	4.38 ± 0.31^{b}	3.38 ± 0.06^{ab}	14.64 ± 0.19^{b}	2.89 ± 0.99^{b}	0.60 ± 0.30^{a}
D4	4.63 ± 0.06^{b}	3.88 ± 0.31^{c}	14.75 ± 0.04^{b}	3.11 ± 1.26^{c}	0.71 ± 0.36^{b}
The interaction effect between the type and dose factors of inoculum					
S1D0	3.00 ± 0.00^{a}	1.00 ± 0.00^{a}	13.33 ± 0.24	$0,00\pm0.00^{a}$	0.00 ± 0.00^{a}
S1D1	3.00 ± 0.00^{a}	2.75 ± 0.43^{b}	13.88 ± 0.43	2.88 ± 0.22^{d}	0.60 ± 0.52^{b}
S1D2	3.00 ± 0.00^{a}	3.00 ± 0.00^{ab}	14.95 ± 0.90	3.38 ± 0.65^{e}	0.65 ± 0.51^{b}
S1D3	3.75 ± 0.43^{b}	3.50 ± 0.50^{b}	14.25 ± 0.15	4.88 ± 0.65^{f}	1.20 ± 0.52^{c}
S1D4	4.50 ± 0.50^{c}	4.50 ± 0.50^{c}	14.83 ± 0.56	5.63 ± 0.65^{g}	1.43 ± 0.13^{c}
S2D0	3.00 ± 0.00^{a}	1.00 ± 0.00^{a}	13.23 ± 0.19	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
S2D1	4.50 ± 0.87^{c}	3.00 ± 0.00^{ab}	15.03 ± 0.58	1.75 ± 0.83^{c}	0.00 ± 0.00^{a}
S2D2	4.75 ± 0.43^{c}	3.00 ± 0.00^{ab}	14.35 ± 0.55	0.43 ± 0.13^{ab}	0.00 ± 0.00^{a}
S2D3	5.00 ± 0.00^{d}	3.25 ± 0.43^{b}	15.03 ± 0.75	0.90 ± 0.60^{b}	0.00 ± 0.00^{a}
S2D4	4.75 ± 0.43^{c}	3.25 ± 0.43^{b}	14.68 ± 0.70	0.60 ± 0.52^{b}	0.00 ± 0.00^{a}

Different superscripts in the same column indicate a very significant effect (p<0.01) used in the fermentation process include bacteria, molds, and yeasts (Ridwan & Haryuni, 2024). The fermentation process in SMW causes an increase in phenol and isoflavone compounds due to anaerobic reactions from the inoculum. Antioxidant activity during fermentation that occurs in SMW involves the role of phenolic compounds. Phenolic compounds can act as antioxidants by breaking the free radical chain directly and capturing various reactive species. High antioxidant content in feed ingredients makes the color of the feed ingredients brighter and low antioxidant content makes the color of the feed ingredients darker (Miksusanti & Elfita, 2012).

Texture

Texture is a characteristic combination of several physical properties including size, shape, quantity and elements that form the material that are felt in the mouth (Hasanah, Haryuni, et al., 2024). In addition to attracting consumer attention to color quality, texture also determines the quality of a product (Ridwan & Haryuni, 2024). The results of statistical analysis showed that the factors of inoculum type, inoculum dose and the interaction between the two factors (inoculum type and dose) had a significant effect ($p<0.01$) on the texture quality of fermented SMW. The texture scores obtained in this study ranged from 1.00-4.50. The texture scores obtained in this study were almost the same as the study Hasanah, Haryuni, et al. (2024) which obtained texture scores from fermented SMW using EM4 of 1.00-3.00. The best SMW texture was obtained by using *Saccharomyces cerevisae* inoculum with a dose of 20%. Fermentation is a complex biochemical process that degrades complex compounds into simple compounds using microorganisms (Rashad, Mahmoud, Abdou, & Nooman, 2011). Changes in the texture of SMW fermented with *Saccharomyces cerevisae* are caused by the degradation of carbohydrates and fibers in SMW by enzymes produced by *Saccharomyces cerevisae*. The fermentation process of SMW with *Saccharomyces cerevisae* causes a depolymerization reaction of complex compounds and the release of derivatives in SMW (Wang et al., 2022). Increasing the dose of *Saccharomyces cerevisae* used in the fermentation process has an impact on increasing metabolism and its ability to degrade substrates which produces ethanol as a by-product (Sitanggang, Sinaga, Wie, Fernando, & Krusong, 2020).

Moisture

The results of statistical analysis showed that the inoculum dose factor had a significant effect ($p<0.01$) on the moisture of fermented SMW, while the type factor and the interaction of the two factors (type and dose of inoculum) had no significant effect ($p>0.05$) on the moisture of SMW. The water content obtained in this study ranged from 13.23-15.03. The lowest moisture of 13.23 was obtained in SMW in the control treatment, while the highest SMW water content of 15.03 was obtained in fermentation using Rhizopus sp. inoculum with a dose of 15%. The moisture in this study was almost the same as the results of the study Hasanah, Haryuni, et al., (2024) which obtained moisture from SMW fermented using EM4 of 12.75-15.10%. The increase in moisture in SMW fermented using *Rhizopus* sp. and *Saccharomyces cerevisae* was due to a decrease in carbohydrate content and an increase in amino acid and fatty acid content (Mok, Tan, Lee, Kim, & Chen, 2019). Several studies have reported that in the SMW fermentation process, microorganisms use it as a carbon source for their metabolism and this increase in metabolism also causes an increase in the water content of SMW (Wang et al., 2022). Solid substrate fermentation (SMW) using *Rhizopus* sp. and *Saccharomyces cerevisae* can increase the conversion of isoflavones from glycoside form to aglycone form. The addition of *Rhizopus* sp. and *Saccharomyces cerevisae* levels makes the conversion of isoflavones greater so that the SMW temperature increases and the water content increases (Nurkhoeriyati & Iswaldi, 2019).

Sugar Concentration

The results of statistical analysis showed that the factors of inoculum type, inoculum dose and interaction between the two factors (inoculum type and dose) had a significant effect ($p<0.01$) on the sugar concentration of fermented SMW. The sugar concentration obtained in this study ranged from 0.00-5.63%. The lowest sugar concentration of 0.00% was obtained in the control treatment, while the highest sugar concentration of 5.63% was obtained in SMW fermented using *Saccharomyces cerevisae* inoculum with a dosage of 20%. The fermentation process using *Saccharomyces cerevisae* yeast is able to utilize carbohydrates and fiber in SMW as a carbon source for its metabolism. Carbohydrates are a source of carbon that functions as a source of energy for microorganisms to survive. In this metabolic process, carbohydrates in SMW will be degraded into simple sugars (Wang et al., 2022). Factors that influence the fermentation process include temperature during the fermentation process, substrate pH, type of inoculum, type of substrate, and the nutrient content of the fermented substrate (Pradipta, Nocianitri, & Permana, 2020).

Alcohol Concentration

The results of statistical analysis showed that the factors of inoculum type, inoculum dose and interaction between the two factors (inoculum type and dose) had a significant effect ($p<0.01$) on the alcohol concentration of fermented SMW. The alcohol concentration obtained in this study ranged from 0.00-1.43%. The lowest alcohol concentration of 0.00% was obtained in the control treatment and SMW fermented using *Rhizopus* sp. inoculum, while the highest alcohol concentration of 1.43% was obtained in SMW fermented using *Saccharomyces cerevisae* with a dose of 20%. Several studies have reported that in the fermentation process, microorganisms will use and utilize carbohydrates and fiber in the substrate as a carbon source for their metabolism, where the by-product of the anaerobic metabolism of these microorganisms is ethanol (alcohol) (Wang et al., 2022). Substrate is a fermentation raw material that contains nutrients needed by microorganisms to grow and then produce fermentation products. The main nutrient for microorganisms to grow and produce fermentation products is carbohydrates. *Saccharomyces cerevisiae* has the ability to degrade complex carbohydrates into simple ones, namely in the form of sugar. *Saccharomyces cerevisiae* can break down sugars from both monosaccharides and disaccharides into ethanol using invertase and zymase enzymes. The invertase enzyme hydrolyzes disaccharides into monosaccharides and the zymase enzyme then converts monosaccharides into alcohol and CO_2 (Hossain, Zaini, & Mahlia, 2017).

CONCLUSION

The results of the study concluded that the interaction between the type and dose of inoculum in fermentation can improve the quality of soy milk waste (SMW). The best quality of soy milk waste was obtained in fermentation with *Saccharomyces cerevisae* at a dose of 15%.

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