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# Effect of the dose and duration of fermentation of catfish waste by a consortium of lipolytic microbes on microbiological quality and nutrient content

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## Abstract

The purpose of the study was to explore the influence of the dose and duration of fermentation of a consortium of microbes, *Pseudomonas aeruginosa, Rhizopus microspores*, and *Candida lipolytica* that resulted in the microbiological quality (total microbes and temperature) and nutrient content (fat and protein content) of the best fermented catfish waste. The research was conducted experimentally in the laboratory using the Complete Random Design of the Nesting Pattern. The treatment consisted of inoculum doses (D1:5%, D2:10%, D3:15%, D4:20%) and fermentation duration (W1:2 days, W2:4 days, W3:8 days) with 3 repeats. The data from the study were tested using multiple fingerprints and continued with the Duncan multiple distance test. The results showed that the inoculum dose exerted a significant effect ($P<0.05$) on total microbes, fat, and protein levels. The fermentation time exerts a noticeable influence ($P<0.05$) on total microbes, temperature, fat, and protein content. The use of a dose of 5% inoculum and a fermentation period of 2 days resulted in the highest number of microbes, which is 216 × 10^9 CFU/mL, the highest protein is 59.15%, and the highest temperature is 30.37 °C. The use of a dose of 10% inoculum and a fermentation period of 2 days resulted in the lowest fat content of 42.46%.

**Keywords:** Catfish Waste; Fermentation; Microbes Lipolytic; Consortium; Nutrient Content

## 1. Introduction

The price of fishmeal is relatively high because it is an imported product. It is necessary to develop domestic fishmeal production to produce fishmeal at a more affordable price and quality according to standards. The raw materials for making local fishmeal can use catfish waste because of its abundant availability. The nutrients contained in catfish waste are lower than those of whole fish. The results of the catfish waste sample test contained 20.94% crude fat and 26.05% crude protein (PT. Saraswanti Indo Genetech, 2022). According to SNI 2715:2013, fishmeal contains a minimum of 50% protein content and a maximum of 12% fat content. It needs to be used optimally so that catfish waste can be used as a feed material for a protein source for livestock. One of the efforts that can be made is by fermentation processing.

Fermentation can improve the economic value and nutritional quality of catfish waste. Fermentation can reduce crude fiber and increase protein. Microorganisms in the fermentation process break down complex elements into simpler elements and increase nutrients, including minerals, vitamins, and antioxidants [1]. Microbes that can be used in the fermentation of catfish waste are a consortium of *Pseudomonas aeruginosa, Rhizopus microsporus*, and *Candida lipolytica*. These microbes produce enzymes that can overhaul the nutrition of catfish waste.

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The success of the fermentation process using a microbial consortium can be known by observing macroscopic values (temperature and number of bacterial colonies). The compatibility parameters of fermented catfish waste with fishmeal according to SNI were measured from nutrition through proximate analysis (crude protein and crude fat). Factors that affect microbial growth include nutrient supply, temperature, water activity, pH value, chemical factors, and irradiation. In addition, each microbe will show different time periods of time needed to grow and adapt, as well as differences in growth patterns. These differences are caused by different adaptation processes [2]. The fermentation process is affected by the time required and the dosage given.

In a study [3], it was shown that the use of 5% kefir grains composed of bacteria and yeast in the manufacture of kefir can reduce fat content with a fermentation time of 2 days. This is in line with research [4] using a 5% dose of kefir grains at 48-hour fermentation can degrade the best crude protein by around 6.00%. Research [5] states that the use of a 5% inoculum dose for chicken feather fermentation can produce many lipase enzymes that can degrade fatty acids and glycerol from fats that can lower crude fat levels. Based on the results of previous research, it can be hypothesized that a consortium of microbes, *Pseudomonas aeruginosa, Rhizopus microsporus*, and *Candida lipolytica,* in the fermentation of catfish waste with an inoculum dose of 5% and a fermentation duration of 2 days, produces the best fermentation quality.

## 2. Research methods

### 2.1. Catfish waste

The amount of catfish waste used is 7.5 kg. Catfish waste consists of head, tail, bones, skin, and fins.

### 2.2. Media creation

The manufacture of media for bacteria, yeast, molds, and consortiums uses Nutrient Agar (NA), Potato Dextrose Agar (PDA), and Aquadest.

#### *2.2.1. Inokulum P. Aeruginosa, R. Microsporus, dan C. Lipolytica (PARMCL)*

PARMCL inoculum is made by fermenting catfish waste with pure culture, broth nutrients, and mineral solutions. The manufacture of PARMCL inoculum was carried out by them.

#### *2.2.2. Mineral solution*

Manufacture of mineral solutions to use $CO(NH_2)_2$, NaCl, $KH_2PO_4$, $MgCl_2$, yeast extract, KCl, $MgSO_4$, $CuSO_4$, and aquades.

#### *2.2.3. Proximate analysis*

For proximate analysis, protein and crude fat tests used $K_2SO_4$, $CuSO_4$, $H_2SO_4$, vaseline, NaOH 40%, Aquades, $H_3BO_3$ 4%, HCl 0.1 N, and $C_6H_{14}$

### 2.3. Research tools

The equipment required in the study is as follows.

- Test tubes serve as a place for diluting samples and breeding grounds for propagated microorganisms.
- Osse functions to take microorganisms that will be reproduced.
- Bunsen functions as a heater so as to create sterilized conditions.
- Glass bottles serve as a place to store inoculum and extracted liquids.
- The glass funnel serves as an aid in transferring liquids.
- The automatic shaker bath functions as an incubation place for inoculum (liquid fermenter) by maintaining a temperature of 35 °C.
- The micropipette functions to introduce microorganisms into the test tube during the dilution process and into the sample.
- The stove serves for heating in proximate analysis and agar making.
- A beaker glass functions to measure the volume of the solution and the place where the solution is heated.
- The sterilizer functions to sterilize equipment.
- An autoclave functions to sterilize objects using high-temperature and high-pressure steam.
- Hammermill functions to grind catfish waste.

- Plastic serves as a place to store fermentation samples.
- Label paper serves to mark the sample jar.
- A magnetic stirrer functions to heat, stir, and homogenize a solution.
- A spatula serves to take samples and chemicals.
- Analytical scales function to weigh chemicals and samples.
- Digital scales function to weigh catfish waste.
- Centrifuge, functions to separate solids and liquids of LIPF.
- Jars serve as fermentation sample holders.
- Clear duct tape serves to glue the lid of the jar.
- The thermometer functions to check the temperature of fermented samples.
- Petri cups function to breed microorganisms whose number will be counted.
- Aluminum foil serves to wrap equipment to be sterilized using an autoclave.
- Syringes serve as a device to transfer liquids.
- Vortex serves to homogenize samples in the test tube.
- The colony counter serves as an aid for calculating microorganisms.
- Kjeldahl pumpkin serves as a tool of destruction in protein analysis.
- Pipettes and filler pipettes serve to dispense chemical solutions.
- A set of distillation tools serves for the analysis of coarse proteins.
- A buret with a 50 mL 0.1 mL scale serves as a titration tool in the analysis of crude proteins.
- An Erlenmeyer flask functions to collect distilled liquids.
- A set of Soxhlet tools functions to extract samples in the analysis of crude fat.
- Filter paper serves as a place for the sample sleeve to be extracted.
- A measuring cup serves to measure the volume of the solution.
- The oven serves to heat and dry samples.
- A desiccant functions to absorb steam in a sample or tool after being ovened.

### 2.4. Research preparation stage

#### *2.4.1. Media creation*

PDA for bacteria. Dissolve 39 g in 1 liter of water. Bring to a boil until completely dissolved. Sterilize with an autoclave at 121 °C for 15 minutes. Stir well before pouring.

Agar nutrients for yeast and mold. Dissolve 28 g in 1 liter of distilled water. Bring to a boil until completely dissolved. Sterilize with an autoclave at 121 °C for 15 minutes.

#### *2.4.2. Manufacture of mineral solutions*

Mineral solution for bacteria.

**Table 1** Mineral Solution Ingredients for Bacteria

| Chemicals | Quantity (g) |
|---|---|
| CO(NH2)2 | 5 |
| NaCl | 5 |
| KH2PO4 | 4 |
| MGCL | 1 |

Dissolve the ingredients with 1 liter of aquods. It was then sterilized using a 121 °C autoclave for 20 minutes [6].

Mineral solution for mold/yeast

**Table 2** Mineral Solution Ingredients for Mold and Yeast

| Chemicals | Quantity (g) |
|---|---|
| Extracts yeast | 5 |
| $CO(NH_2)_2$ | 5 |
| KCl | 0.5 |
| $MgSO_4$ | 0.5 |
| $CuSO_4$ | 0.001 |

Dissolve the ingredients with 1 liter of aquods. It was then sterilized using a 121 °C autoclave for 20 minutes [6].

*2.4.3. Inoculum manufacturing*

*Pseudomonas aeruginosa*, Rhizopus microspores, and Candida lipolytica are obtained from pure culture. The procedure for making the inoculum is as follows.

Inoculum bacterium

Weigh the ingredients according to the amount needed, according to the table.

**Table 3** Materials for Making Bacterial Inoculum

| Chemicals | Quantity |
|---|---|
| Catfish sterile | 1 g |
| Nutrient broth | 1g |
| Laputan mineral | 90 mL |
| Bacteria | 10 mL |

Catfish are ground and sterilized using a 12 °co. autoclave for 20 minutes. After sterilization, catfish, nutrient broth, and mineral solution are mixed and stirred using a magnetic stirrer. After mixing evenly, add bacterial culture to the test tube to which 10 mL has been added so that the volume is 100 Ml. Then it was incubated for 4 days at a temperature of 50 °C in a shaker bath (primary inoculum). Furthermore, dilution was carried out 10 times by adding a sterile mineral solution (secondary inoculum) [7].

Inoculum yeast

Weigh the ingredients according to the amount needed, according to the Table 4.

**Table 4** Mold Inoculum Manufacturing Materials/Yeast

| Chemicals | Quantity |
|---|---|
| Catfish sterile | 1 g |
| Nutrient broth | 1 g |
| Laputan mineral | 90 mL |
| yeast | 10 mL |

Catfish are ground and sterilized using a 12 °C autoclave for 20 minutes. After sterilization, catfish, nutrient broth, and mineral solvents are mixed and stirred using a magnetic stirrer. After mixing evenly, add bacterial culture to the test tube to which 10 mL has been added so that the volume is 100 mL. Then it is incubated for 3 days at 35 °C in a shakerbath (primary inoculum). Furthermore, dilution was carried out 10 times by adding a sterile mineral solution (secondary inoculum) [7].

### 2.5. Stages of fermentation of catfish waste with microbial consortiums

- The ground catfish waste is weighed as much as 200 grams and put into a heat-resistant plastic.
- Make a total of 36 samples (3 days, 4 doses, and 3 replicas). Sample in a 121 °C autoclave for 15 minutes.
- Remove the liquid from the plastic sample LIPF.
- Fill the sample by dose (5%, 10%, 15%, and 20%).
- Store the sample in a jar and label it according to the dose, repeat, and fermentation time
- The samples are fermented in a 35 °C temperature fermenter cabinet for 2, 4, and 8 days.

**Table 5** Use of Fermentation Ingredients Each Time Treatment

| No. | Ingredients | Dosage | | | |
|---|---|---|---|---|---|
| | | **5%** | **10%** | **15%** | **20%** |
| 1. | Catfish Waste | 200 g | 200 g | 200 g | 200 g |
| 2. | Inoculum seconds PARMCL | 10 mL | 20 mL | 30 mL | 40 mL |

### 2.6. Observed variables and measurements

*2.6.1. Microbiological qualities*

Temperature Data Capture

Temperature data measurement using a thermometer is measured at 3 sample points, namely, the side and center of the sample. The results of the measurement of 3 temperature points are averaged with the following formula:

$$\bar{x} = \frac{\sum x_1}{n}$$

Description

- $\bar{x}$ = Average sample
- $x_1$ = Sample data
- $n$ = Number of samples taken

*2.6.2. Total microbial analysis*

Total microbial analysis uses the Total Plate Count (TPC) method. The fermented samples were examined by TPC by diluting 1 g of the sample 9 times. Samples were cultured in the medium and counted after 4 days using colony counters. After incubation, calculate the number of colonies that grow and multiply them by their dilution factor [8].

$$Colony\ forming\ units = \frac{Jumlah\ koloni}{Faktor\ pengenceran} \times \frac{1}{Faktor\ pengenceran(10^1)}$$

*2.6.3. Nutrient content*

*Crude protein*

- Analysis of crude proteins using the Kjeldahl method.
- Weighing of the mashed sample by 1 g.
- Filling the sample into a Kjeldahl flask.
- Weighing 7 g of $K_2SO_4$ and 3 g of $CuSO_4$
- Addition of 7 g of K2SO4 and 3 g of CuSO4 to the sample of the Kjeldahl flask.
- The addition of a solution of $H_2SO_4$ of 12 mL is carried out in an acidic cabinet.
- The destruction process is carried out in the acid chamber by heating the samples in the Kjeldahl pumpkin using an electric stove until it is tosca green.
- Refrigerate the Kjeldahl pumpkin by letting it sit for 20 minutes.
- Addition of 25 mL of aqueducts to the Kjeldahl flask containing the sample.
- The addition of 50 mL of 40% NaOH and a few boiling stones to the Kjeldahl flask containing the sample.

- The addition of 30 mL of $H_3BO_3$ 4% to the Erlenmeyer with the addition of a 7-drop BCG-MR indicator to capture the distillate from the distillation results.
- Distillation device circuitry.
- The distillate obtained from the distillation results is titrated using a standard solution of HCl 0.1 N until the color of the solution changes to pale pink.

The calculation of Crude Protein (CP) is carried out using the following formula

$$CP = \frac{(ml\ HCl\ used \times Normalitas\ HCl \times 0{,}14 \times 6{,}25)}{sample\ weight} \times 100\%$$

*2.6.4. Crude fat*

Fat content analysis using the Soxhlet method.

- The fat casings to be used are dried in an oven at 105 °C for 1 hour
- The fat sleeve is cooled in a desiccant for 15 minutes and weighed (W1)
- Samples of ± 1 gram were mashed and wrapped using filter paper formed into a thimble and then weighed (W2)
- The sample was then put into Soxhlet, to which hexane solvent was added for 11/2 cycles
- Extraction is carried out for ± 6 hours until the solvent descends back through the siphon into a clear-colored fat flask
- The sample that has been separated with hexane is then heated in the oven at 105 °C for 1 hour
- Samples are cooled in a desiccant for 15 minutes and weighed (W3)
- The calculation of Crude Fat (LK) is carried out using the following formula

$$\%lemak = \frac{W2 - W3}{W2 - W1} \times 100\%$$

- W1 = Selongsong + Hekter
- W2 = Sample weight + Casing + Initial Hectare (grams)
- W3 = Sample weight + Sleeve + Final Hectare (g)

*2.6.5. Statistical Analysis*

This research was conducted experimentally in the laboratory using the Complete Random Design of Nested Pattern method with a dose factor consisting of 4 levels nested in the fermentation time factor consisting of 3 levels. Each treatment is repeated 3 times. The combination of catfish waste fermentation treatment with the use of a consortium dose of *P. aeruginosa, R. microspores*, and *C. lipolytic* (PARMCL) and fermentation time is as follows:

- D1W1 = Fermented with PARMCL 5% for 2 days
- D1W2 = Fermentation with 5% PARMCL for 4 days
- D1W3 = Fermentation with 5% PARMCL for 8 days
- D2W1 = Fermentation with PARMCL 10% for 2 days
- D2W2 = Fermentation with PARMCL 10% for 4 days
- D2W3 = Fermentation with PARMCL 10% for 8 days
- D3W1 = Fermentation with PARMCL 15% for 2 days
- D3W2 = Fermentation with PARMCL 15% for 4 days
- D3W3 = Fermentation with PARMCL 15% for 8 days
- D4W1 = Fermentation with PARMCL 20% for 2 days
- D4W2 = Fermentation with PARMCL 20% for 4 days
- D4W3 = Fermentation with PARMCL 20% for 8 days

To determine the degree of difference between each treatment using the PARMCL Dose, it was tested using the Duncan multiple spacing tests [8].

## 3. Results and discussion

### 3.1. Effect of treatment on the microbiological quality of LIPF

*3.1.1. Total Microbes*

Total microbes are one of the indicators to assess the effectiveness of the fermentation process. The high number of microbes can also speed up the time required for the fermentation process. The total microbes from fermented catfish waste research are presented in Table 6.

**Table 6** Effect of treatment on total microbes

| **Treatment** | | **Detestation** | | | **Average** | **Average** |
|---|---|---|---|---|---|---|
| Dosage | Time | 1 | 2 | 3 | | |
| .....× $10^9$ CFU/ML..... | | | | | | |
| $D_1$ | $W_1$ | 296 | 156 | 196 | 216 | 88.00 |
| | $W_2$ | 6 | 11 | 7 | 8 | |
| | $W_3$ | 36 | 46 | 38 | 40 | |
| $D_2$ | $W_1$ | 29 | 40 | 38 | 35.67 | 20.11 |
| | $W_2$ | 25 | 19 | 22 | 22 | |
| | $W_3$ | 2 | 3 | 3 | 2.67 | |
| $D_3$ | $W_1$ | 25 | 19 | 27 | 23.67 | 28.11 |
| | $W_2$ | 70 | 34 | 40 | 48 | |
| | $W_3$ | 15 | 9 | 14 | 12.67 | |
| $D_4$ | $W_1$ | 124 | 76 | 88 | 96 | 52.56 |
| | $W_2$ | 10 | 5 | 8 | 7.67 | |
| | $W_3$ | 63 | 41 | 58 | 54.00 | |

Description

| | |
|---|---|
| D1 = Inoculum PARMCL 5% dose | W1 = Fermentation Time 2 Days |
| D2 = Inoculum PARMCL 10% dose | W2 = Fermentation Time 4 Days |
| D3 = Inoculum PARMCL 15% dose | W3 = Fermentation Time 8 Days |
| D4 = Inoculum PARMCL 20% dose | |

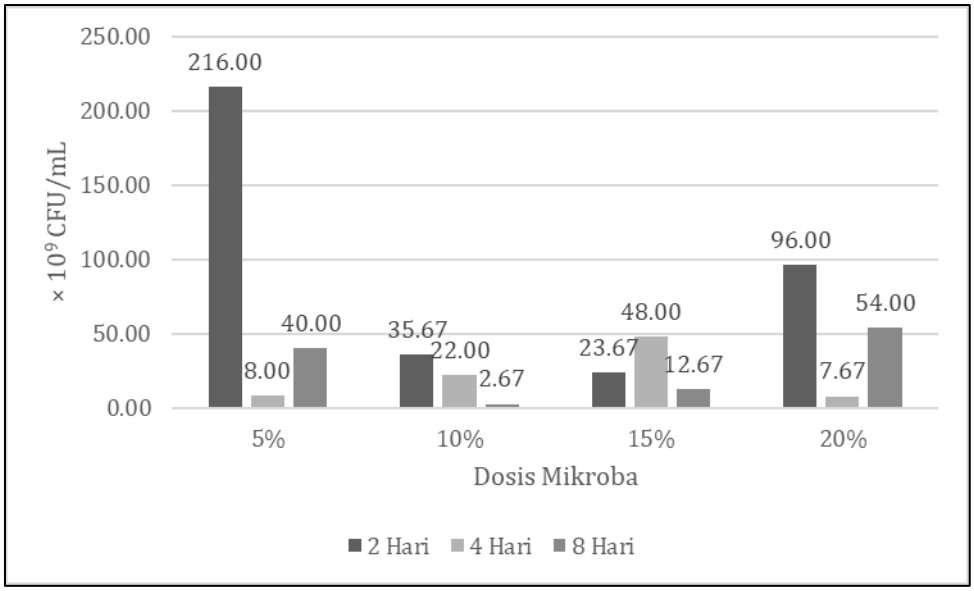


**Figure 1** Total microbial waste of fermented catfish

Based on Illustration 1, it can be seen that the total LIPF microbes on average decreased on day 4. Based on the results of the variegated fingerprint test, it was shown that the administration of inoculum dose and the length of time LIPF had a significant effect ($P<0.05$) on the total microbes. The Duncan Multiple Distance Test was performed to determine the difference in the average total microbes between the treatments presented in Table 7.

**Table 7** Duncan distance tests the effect of inoculum dosing on the number of microbes

| Treatment | Average (× $10^9$ CFU/mL) | Significance |
|---|---|---|
| $D_1$ | 88.00 | c |
| $D_2$ | 20.11 | a |
| $D_3$ | 28.11 | b |
| $D_4$ | 52.56 | bc |

Description = D1 (5% PARMCL inoculum dose), D2 (10% PARMCL inoculum dose%), D3 (15% PARMCL inoculum dose), D4 (20% PARMCL inoculum dose). Different letters in the significance column show a noticeable difference ($p<0.05$).

Table 7 shows the difference in the treatment of temperature. The effect of 5% inoculum (D1) dose was not significantly different ($P>0.05$) compared to the use of 10% inoculum dose (D2), but significantly different ($P<0.05$) from the use of 15% inoculum dose (D3) and 20% inoculum dose (D4). The use of a dose of 10% inoculum (D2) was not significantly different ($P>0.05$) compared to the use of a dose of 20% inoculum (D4). A 5% inoculum dose and a 20% inoculum dose have faster total microbial growth, which can result from being in an exponential phase. Where these microbes have adapted to the available substrate so that the process of cell multiplication increases [9].

Doses of inoculum that are too high can result in microbial metabolism being disrupted and decreased. This is because the availability of dissolved oxygen is reduced, resulting in competition between microbes [9]. This causes microbes to reach the stationary phase faster due to nutrient deficiencies [10]. The following was performed: the Duncan Multiple Distance Test to determine the difference in the average total microbes between the fermentation times presented in Table 8.

**Table 8** Distance test Duncan effect of fermentation length on microbial count

| Treatment | Average (× $10^9$ CFU/mL) | Significance |
|---|---|---|
| $D_1W_1$ | 216 | c |
| $D_1W_2$ | 8 | a |
| $D_1W_3$ | 40 | b |

Remarks: D1 (PARMCL inoculum dose 5%), W1 (Fermentation Time 2 days), W2 (Fermentation Time 4 days), W3 (Fermentation Time 8 days). Different letters in the significance column show a noticeable difference ($p<0.05$).

Table 8 shows the difference in the treatment of total microbes. The effect of 5% inoculum dose of 2-day fermentation time (D1W1) was significantly different ($P<0.05$) from the 5% inoculum dose of 4-day fermentation (D1W2), and the inoculum dose of 5% 8-day fermentation (D1W3) was not significantly different ($P>0.05$) from the dose.

The average total microbes decreased. Degradation can result from a reduced amount of substrate. These microbes multiply and grow using the nutrients available in the substrate. Microbes on day 4 tend to be lower than on day 2 because the longer the fermentation, the fewer nutrients in the medium are, so that there is competition between microbes, and some enter the death phase [11]. Fermentation times that exceed the optimal limit will cause microbes to approach the death phase ([12]. The total decrease in microbes can also be caused by too high temperatures that inhibit cell growth [13]. In addition, the longer the fermentation process, the more acidic the environmental conditions. It can reduce the number of microbes. PARMCL microbes tend to grow in environments with neutral pH. A drop in pH will inhibit the growth of microbes that are not resistant to acidic conditions [14].

There are several possibilities of fluctuating microbial volumes. Among them can be caused by interactions between competing microbes. The number of microbes is increasing, and the rapid decrease can result from the interaction of one microbe with another microbe [15]. The competition that occurs can disrupt the growth of these microbes. According to the opinion [15], competition shows the existence of negative interactions between microbes, which will affect their life and growth.

The number of microbes increases again on day 8, which can be caused by microbes growing and developing. Microbes grow in different life cycles and nutrient needs [16]. Each type of microbe has a specific growth time span, so that during growth, there are microbes whose growth decreases, and one or more other microbes increase [14].

A dose of 5 % inoculum with a fermentation duration of 2 days (D1W1) yielded the best combination. This can be caused by the balance between nutrients in the available substrate. As stated [10], the limiting factors for microbial growth are secondary metabolic accumulation and nutrient availability in the substrate.

*3.1.2. Temperature*

Temperature is also one of the indicators of fermentation success. An increase in temperature in the fermentation process indicates the metabolic activity of microbes. In the fermentation process, energy is released by microbes in the form of heat as a by-product. The higher the temperature of the substrate in the fermentation process, the higher the microbial activity in remodeling the substrate. The temperature of the research results of fermented catfish waste is presented in Table 9.

**Table 9** Effect of treatment on temperature

| Treatment | | Detestation | | | Average | Average |
|---|---|---|---|---|---|---|
| Dosage | Time | 1 | 2 | 3 | | |
| .....°C..... | | | | | | |
| $D_1$ | $W_1$ | 29.5 | 31.3 | 30.30 | 30.37 | 30.78 |
| | $W_2$ | 31.63 | 31.87 | 31.50 | 31.67 | |
| | $W_3$ | 30.23 | 30.5 | 30.22 | 30.32 | |
| $D_2$ | $W_1$ | 29.83 | 31.67 | 30.75 | 30.75 | 30.43 |
| | $W_2$ | 30.63 | 30.57 | 31.40 | 30.87 | |
| | $W_3$ | 29.87 | 29.6 | 29.59 | 29.69 | |
| $D_3$ | $W_1$ | 30.83 | 31.63 | 30.48 | 30.98 | 31.27 |
| | $W_2$ | 33.47 | 33.43 | 33.30 | 33.40 | |
| | $W_3$ | 29.53 | 29.42 | 29.30 | 29.42 | |
| $D_4$ | $W_1$ | 33.27 | 30.2 | 28.93 | 30.80 | 30.49 |
| | $W_2$ | 31.77 | 31.4 | 31.93 | 31.70 | |
| | $W_3$ | 28.93 | 29.11 | 28.90 | 28.98 | |

Description

| | |
|---|---|
| D1 = Inoculum PARMCL 5% dose<br>D2 = Inoculum PARMCL 10% dose<br>D3 = Inoculum PARMCL 15% dose<br>D4 = Inoculum PARMCL 20% dose | W1 = Fermentation Time 2 Days<br>W2 = Fermentation Time 4 Days<br>W3 = Fermentation Time 8 Days |

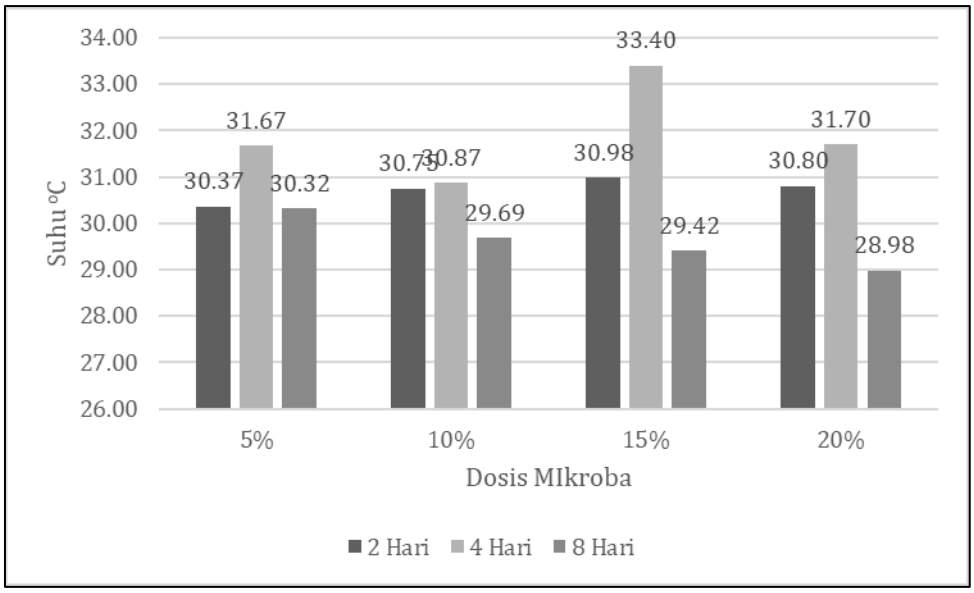


**Figure 2** Temperature of fermented catfish waste

Based on Illustration 2, it can be seen that the temperature of the LIPF increased on day 4 and decreased on day 8. Based on the results of the variety, it showed that the administration of inoculum doses did not have a real effect (P>0.05) on temperature, but the fermentation time had a real effect (P<0.05) on fermentation temperature. The Duncan Multiple Distance Test was carried out to determine the difference in average fermentation temperature between the fermentation time and the smallest dose, which is 5% (D1), presented in Table 10.

**Table 10** Duncan Distance Test: Effect of Fermentation Length on Temperature

| Treatment | Average | Significance |
|---|---|---|
| .....$^{o}$C..... | | |
| $D_1W_1$ | 30.37 | ab |
| $D_1W_2$ | 31.67 | b |
| $D_1W_3$ | 30.32 | a |

Remarks: D1 (PARMCL inoculum dose 5%), W1 (Fermentation Time 2 days), W2 (Fermentation Time 4 days), W3 (Fermentation Time 8 days). Different letters in the significance column show a real difference (p<0.05)

Table 10 shows the difference in the treatment of fermentation temperature. The effect of 5% inoculum dose 4 days of fermentation (D1W2) was not significantly different (P>0.05) from a 5 % inoculum dose of 2 days of fermentation (D1W1), but there was a real effect (P<0.05) with a 5% inoculum dose of 8 days of fermentation (D1W3). The effect of inoculum administration of 5% of the 2-day fermentation period (D1W1) was not significantly different (P>0.05) from the 5% inoculum dose of 8-day fermentation (D1W3). The best treatment that produces the highest temperature is the treatment of administering a dose of 5 % inoculum for a 2-day fermentation duration (D1W1) of 30.37 $^{o}$C.

The results showed that the temperature during fermentation ranged from 28.98-33.4 °C. The temperature corresponds to the optimal temperature of PARMCL microbes. *P. aeruginosa* grows optimally at 25–35 °C, *R. microsporus* grows at 25–55 °C, and *Candida lipolytica* grows optimally between 28–34 °C [17–20]. There is a change in temperature during fermentation that indicates the fermentation process is going well [21]. According to [22], during the fermentation process, it produces alcohol, lactic acid, acetic acid, and an increase in temperature. According to [23], temperature can affect microbial activity in two ways. When the temperature increases, the metabolic rate increases, and growth accelerates. On the other hand, when the temperature decreases, metabolism and growth slow down

The fermentation temperature on the 4th day increased. The increase is due to the microbial activity of PARMCL when overhauling complex elements into simple. The overhaul is an exothermic reaction that gives off heat [24]. An increase in temperature in the fermentation process can be caused by the activity of microbes in decomposing organic matter and growing rapidly [25]. Temperature increases as microbes give off heat when decomposing carbohydrates [26–27]. Microbes in the fermentation process passively decompose carbohydrates [28]. The fermentation time also affects the fermentation temperature. The longer the fermentation process, the more organic elements are absorbed, and the organic acid content increases. This will result in a decrease in pH and an increase in temperature [29].

An increase in temperature will lead to an increase in chemical reactions. This is because the increase in temperature will cause the reactant energy to increase [30]. Under certain conditions, after the optimal temperature, the increase in temperature is not in sync with microbial growth. This is due to the stability between the protein structures of the enzyme, which is affected by the increase in temperature. A much higher temperature increase will inhibit cell growth, which will then significantly reduce fermentation activity.

On the 8th day, the average temperature decreased. Temperature drops also occurred in the study [25]. When entering the end of fermentation, the temperature will decrease from the optimal temperature during the fermentation process. The decrease in temperature can be caused by the nutrient content in the fermentation substrate having been reduced or decomposed [31-32]. When the temperature drops, there is also a decrease in enzyme activity. This can be caused by denatured proteins or substrates that undergo conformational changes.

### 3.2. Effect of treatment on the nutrient content of fermented catfish waste

*3.2.1. Extract ether/ fat*

Fat is made up of oxygen, hydrogen, and carbon. The carbon and hydrogen content in fat is higher than the oxygen content. Energy in fat is 2.25 times more than in carbohydrates. Fat content after fermentation indicates the result of an overhaul by microbes. Lipolytic enzymes in microbes are expected to reduce fat levels in catfish waste. The best fermentation dosage and length result in the lowest fat content. The fat content of fermented catfish waste is presented in Table 11.

**Table 11** Effect of treatment on fat levels

| **Treatment** | | **$W_0$** | **Average** | | | **Average** | **Average** |
|---|---|---|---|---|---|---|---|
| Dosage | Time | | 1 | 2 | 3 | | |
| .....%..... | | | | | | | |
| $D_1$ | $W_1$ | 39.10 | 43.38 | 48.83 | 46.93 | 46.38 | 52.48 |
| | $W_2$ | | 54.38 | 56.89 | 56.40 | 55.89 | |
| | $W_3$ | | 57.09 | 51.25 | 57.17 | 55.17 | |
| $D_2$ | $W_1$ | | 41.46 | 43.36 | 42.57 | 42.46 | 47.67 |
| | $W_2$ | | 50.30 | 53.53 | 53.75 | 52.53 | |
| | $W_3$ | | 49.33 | 47.02 | 47.71 | 48.02 | |
| $D_3$ | $W_1$ | | 30.08 | 28.24 | 29.41 | 29.24 | 43.77 |
| | $W_2$ | | 55.95 | 54.99 | 58.32 | 56.42 | |
| | $W_3$ | | 48.55 | 41.05 | 47.36 | 45.65 | |
| $D_4$ | $W_1$ | | 48.05 | 45.00 | 47.08 | 46.71 | 54.01 |
| | $W_2$ | | 57.40 | 57.56 | 57.64 | 57.53 | |
| | $W_3$ | | 62.05 | 52.44 | 58.91 | 57.80 | |

Description

| | |
|---|---|
| D1 = Inoculum PARMCL 5% dose | P0 = non-fermentation |
| D2 = Inoculum PARMCL 10% dose | W1 = Fermentation Time 2 Days |
| D3 = Inoculum PARMCL 15% dose | W2 = Fermentation Time 4 Days |
| D4 = Inoculum PARMCL 20% dose | W3 = Fermentation Time 8 Days |

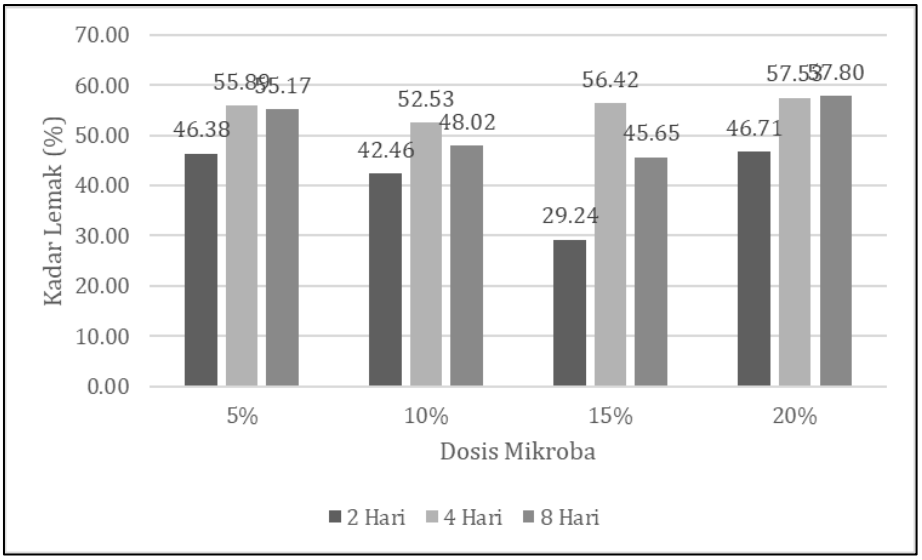


**Figure 3** Fat content of fermented catfish waste

Based on the results of the variety, it was shown that the administration of inoculum dose and the length of time LIPF had a significant effect (P<0.05) on LIPF fat levels. In the D3W1 treatment, there was a decrease in the crude fat content of non-fermented fat by 9.86%. The Duncan Multiple Distance Test was performed to determine the difference in average fat content between the treatments presented in Table 12.

**Table 12** Distance test Duncan effect of inoculum dose on fat content

| Treatment | Average | Significance |
|---|---|---|
| .....% ..... | | |
| $D_1$ | 52.48 | b |
| $D_2$ | 47.67 | a |
| $D_3$ | 43.77 | a |
| $D_4$ | 54.01 | b |

Description = D1 (5% PARMCL inoculum dose), D2 (10% PARMCL inoculum dose%), D3 (15% PARMCL inoculum dose), D4 (20% PARMCL inoculum dose). Different letters in the significance column show a real difference (p<0.05)

Table 12 shows the difference in the treatment of fat content. The effect of giving a dose of 15% inoculum (D3) was not significantly different (P>0.05) from that of a 10% inoculum dose (D2), but had a real effect (P<0.05) with a dose of 20% inoculum (D4) and a dose of 5% inoculum (D1). The Duncan Multiple Distance Test was performed to determine the difference in average fat content between fermentation times presented in Table 13.

**Table 13** Distance test Duncan effect of fermentation length on fat content

| Treatment | Average | Significance |
|---|---|---|
| .....% ..... | | |
| $D_2W_1$ | 42.46 | a |
| $D_2W_2$ | 52.53 | c |
| $D_2W_3$ | 48.02 | b |

Description: D3 (15% PARMCL inoculum dose), W1 (2 days fermentation), W2 (4 days fermentation), W3 (8 days fermentation). Different letters in the significance column show a real difference (p<0.05)

Table 13 shows the difference in the treatment of LIPF fat levels. The effect of inoculum dose 10% of the fermentation period of 2 days (D2W1) was significantly different (P>0.05) compared to the inoculum dose of 10% of the fermentation period of 8 days (D2W3) and the inoculum dose of 15% of the fermentation period of 4 days (D2W2). The best treatment that produced the lowest fat content was the treatment of giving a dose of 15% inoculum and a fermentation duration of 2 days (D3W1) of 29.24%. Fat levels increase or decrease due to the degradation process of fatty acids from the activity of microbial secretion.

Fat levels on day 4 showed an improvement. An increase in fat indicates the presence of fatty acid formation activity in the substrate. As per the statement [33], fat levels increase due to the metabolic processes present in the substrate. Opinion [34], the high activity of microbes results in high fatty acids that can increase the crude fat content. A consortium of microbes degrades organic matter to form fat so that fat levels increase [35]. The resulting lipids will be used to meet the living needs of microbes. Research [36] on the fermentation of chicken feather flour *by B. subtilis* can increase crude fat by 5.25%. This is due to the balance of concentration between the fermentation time and the inoculum dose, with the amount of substrate.

Phospholipids in microbes are also the cause of increased fat levels. According to [37], the crude fat content in the mass of microbial cells that grow and develop in the substrate during fermentation. Decomposing microbial cells can cause fat levels to increase. Crude fat increases because it is composed of substances that are difficult to dissolve in water, including ether, chloroform, and benzene [38]. During the fermentation process, the content of dry matter also decreases, so there is an increase in the fermented product [39].

There was a decrease in crude fat content on the 8th day of fermentation. Fat levels decrease due to the activity of a consortium of microbes that produce the enzyme lipase, and fat is used as a nutrient for its metabolic activity. There is a decrease in fat levels due to the fat consumed by microbes for metabolism, especially lipolytic microbes [40]. According to [39], fat content decreases at the beginning of fermentation due to the presence of microbes that use fat for growth. Some of the fatty acids formed also undergo evaporation, resulting in a decrease in fat levels [36].

The resulting lipase enzyme hydrolyzes the fat into free fatty acids and glycerol. These free fatty acids are easily damaged, which results in a decrease in fat levels. Complex triglyceride bonds are broken down into simple bonds by lipase enzyme types 1-3 [41]. The longer the fermentation, the more lipase enzymes are produced, so that more crude fat content is degraded into fatty acids. The inoculum dose of 15% and the fermentation time of 2 days (D3W1) resulted in the lowest average crude fat content of LIPF, so D3W1 was the best dose to produce the lowest fat content compared to other times and doses. A fermentation period of 2 days can produce products with the lowest fat content, which is suspected because the fermentation period is the growth time of microbial consortiums so that it can produce large amounts of lipase enzymes and spur a decrease in fat content [36].

### 3.3. Up to protein

Protein is a macromolecule that contains amino acids that have important functions in the body, including building and regulating substances. Proteins are made up of the elements nitrogen, carbon, oxygen, and hydrogen. Nitrogen is the main element in protein. Protein levels after fermentation show the result of an overhaul by microbes. Proteolytic enzymes in microbes are expected to hydrolyze proteins in catfish waste. The best dosage and fermentation time result in the highest protein levels. The protein content of fermented catfish waste is presented in Table 14.

**Table 14** Effect of treatment on protein levels

| **Treatment** | | **$W_0$** | **Average** | | | **Average** | **Average** |
|---|---|---|---|---|---|---|---|
| Dosage | Time | | 1 | 2 | 3 | | |
| ……%…… | | | | | | | |
| $D_1$ | $W_1$ | 28.12 | 59.78 | 58.45 | 59.21 | 59.15 | 47.07 |
| | $W_2$ | | 36.98 | 32.80 | 37.74 | 35.84 | |
| | $W_3$ | | 42.92 | 50.63 | 45.12 | 46.22 | |
| $D_2$ | $W_1$ | | 50.18 | 49.92 | 50.02 | 50.04 | 46.37 |
| | $W_2$ | | 44.53 | 34.53 | 36.53 | 38.53 | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | $W_3$ | | 52.71 | 47.19 | 51.75 | 50.55 | |
| $D_3$ | $W_1$ | | 60.27 | 64.27 | 61.52 | 62.02 | 44.28 |
| | $W_2$ | | 31.30 | 29.66 | 29.13 | 30.03 | |
| | $W_3$ | | 38.88 | 43.80 | 39.69 | 40.79 | |
| $D_4$ | $W_1$ | | 37.11 | 35.36 | 36.39 | 36.29 | 33.06 |
| | $W_2$ | | 24.46 | 33.61 | 28.43 | 28.83 | |
| | $W_3$ | | 33.23 | 36.21 | 32.77 | 34.07 | |

Description

| | |
|---|---|
| D1 = Inoculum PARMCL 5% dose | P0 = non-fermentation |
| D2 = Inoculum PARMCL 10% dose | W1 = Fermentation Time 2 Days |
| D3 = Inoculum PARMCL 15% dose | W2 = Fermentation Time 4 Days |
| D4 = Inoculum PARMCL 20% dose | W3 = Fermentation Time 8 Days |

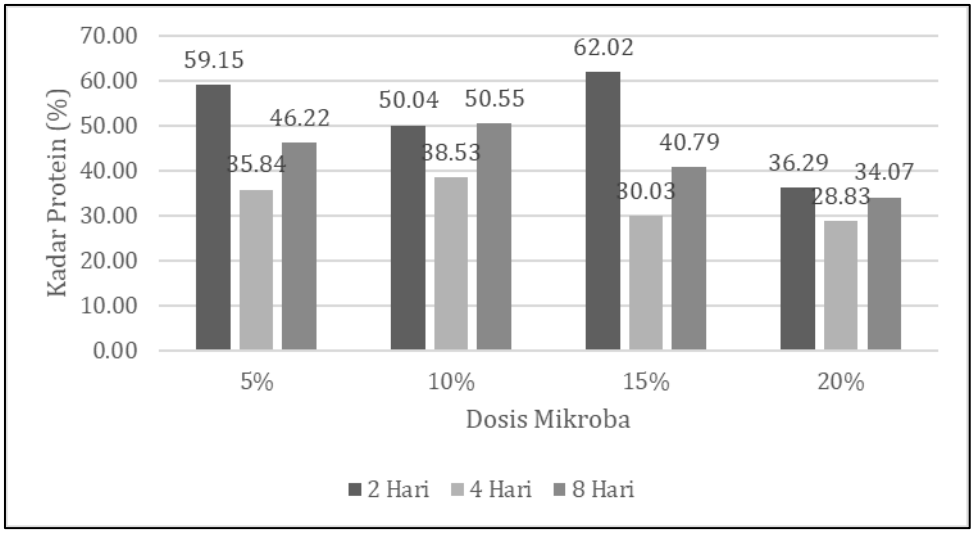


**Figure 4** Protein content of fermented catfish waste

Based on Illustration 4, it can be seen that the level of LIPF protein decreases on day 4 and increases on day 8. Based on the results of the variety, it was shown that the dose of inoculum and the length of time of LIPF had a significant effect ($P<0.05$) on the protein content. The Duncan Multiple Distance Test was performed to determine the difference in average protein content between the treatments presented in Table 15.

**Table 15** Distance test Duncan effect of inoculum dosing on protein levels

| Treatment | Average | Significance |
|---|---|---|
| .....% ..... | | |
| D1 | 47.07 | b |
| D2 | 46.37 | b |
| D3 | 44.28 | b |
| D4 | 33.06 | a |

Description = D1 (5% PARMCL inoculum dose), D2 (10% PARMCL inoculum dose%), D3 (15% PARMCL inoculum dose), D4 (20% PARMCL inoculum dose). Different letters in the significance column show a real difference ($p<0.05$)

Table 15 shows the difference in the treatment of LIPF fat levels. The effect of administering a dose of 5% inoculum (D1) was not significantly different ($P>0.05$) from that of a 10% inoculum dose (D2) and a dose of 15% inoculum (D3), but had a real effect ($P<0.05$) with a dose of 20% inoculum (D4).

The average protein content decreases with the addition of doses. Giving a dose with a certain high limit can reduce the protein content of the substrate. This is because the higher the inoculum dose, the more microbes there are and the more enzymes produced. Many nutrients are required to meet the needs of these microbes, resulting in an imbalance between enzymes and available substrates [36]. The Duncan Multiple Distance Test was performed to determine the difference in average protein levels between the treatments presented in Table 16.

**Table 16** Distance test Duncan effect of fermentation length on protein levels

| Treatment | Average | Significance |
|---|---|---|
| .....% ..... | | |
| $D_1W_1$ | 59.15 | c |
| $D_1W_2$ | 35.84 | a |
| $D_1W_3$ | 46.22 | b |

Remarks: D1 (PARMCL inoculum dose 5%), W1 (Fermentation Time 2 days), W2 (Fermentation Time 4 days), W3 (Fermentation Time 8 days). Different letters in the significance column show a real difference ($p<0.05$)

Table 16 shows the difference in treatment of LIPF fat levels. The effect of 5% of the 2-day fermentation period (D1W1), the 5% inoculum dose of 4-day fermentation (D1W2), and the 5% inoculum dose of 8-day fermentation (D1W3) had a real effect ($P<0.05$). The best treatment that produced the highest protein levels was 5% inoculum treatment for 2 days of fermentation (D1W1) of 59.15%. Wahyuni's (2023) research on the fermentation of chicken feathers using *B. subtilis* also produces the best crude protein with the use of a dose of 5% inoculum.

The crude protein content in fermented catfish waste increased on average on days 2 and 8 compared to non-fermented catfish. Protein levels are increased due to the activity of protease enzymes produced by PARMCL microbes. The results of the study [42] explain that the protease enzyme produced by *A. niger* can increase the value of crude protein by 8.36%. The PARMCL microbial consortium produces protease enzymes that can break down proteins from substrates.

The protease enzyme will convert insoluble proteins into soluble proteins. Proteins are remodeled into polypeptides, then into simple peptides, which are then amino acids. These amino acids will be used by microbes to grow and develop [43]. This causes the amount of microbial biomass to increase. Microbes themselves are single-cell proteins, which of course, will indirectly increase the protein levels of the substrate. The single-cell protein contributed by microbes is quite high, which is about 40-65% protein [44].

During the fermentation process, the microbes will secrete enzymes, which are proteins, and the microbes themselves are also the source of single-cell proteins. Microbial activity in the fermentation process is influenced by the nutrients contained or added in the fermentation media of the microbial substrate. The microbial activity is to degrade proteins into amino acids, peptides, and volatile nitrogen compounds such as ketones and ammonia [45]. The longer the fermentation time, the more protein is reduced as a result of the prolonged activity of proteolytic bacteria that break down proteins [46]. In addition, the increase in protein after fermentation can also be caused by microbes converting inorganic nitrogen from urea to organic nitrogen [42]. The nitrogen will be fixed in the substrate and measured as a crude protein [47]. Molds in microbes also contain nucleic acids that can provide increased protein levels.

On the 4th day of fermentation, the average protein level decreases. Protein levels decreased allegedly because PARMCL microbes utilize substrate proteins to live [48]. During the fermentation process, nitrogenous gas, namely ammonia, will evaporate, which causes the protein content to decrease. Too long fermentation times can also result in decreased microbial activity, leading to protein degradation [49].

Decreased protein levels can also be caused by the degradation of protein structure or proteolysis of collagen into proline hydroxy, which produces protein fragments with shorter peptide chains. Protein decreases as more proteolysis occurs because more and more soluble proteins are produced [50].

## 4. Conclusion

Based on the results of observations and discussions, the following conclusions can be obtained:

- The use of inoculum doses exerts a noticeable effect on total microbes, fat, and protein levels, but does not affect temperature. The fermentation time exerts a noticeable influence on the total microbes, temperature, fat, and protein content.
- The use of a dose of 5% inoculum and a fermentation period of 2 days resulted in the highest number of microbes at 216 109 CFU/mL, the highest protein at 59.15%, and the highest temperature at 30.37 °C. The use of a dose of 10% inoculum and a fermentation period of 2 days resulted in the lowest fat content of 42.46%.

## Compliance with ethical standards

*Disclosure of conflict of interest*

No conflict of interest is to be disclosed

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