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The effect of local soybean fermentation duration using yeast on total plate count, temperature, ph, and water content

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Abstract

This research aims to determine the best use of yeast and fermentation time for Total Plate Count (TPC), temperature, pH, and water content in fermented local soybeans. This research used a Completely Randomized Design with a nested pattern with two factors. Factor 1 uses yeast *Saccharomyces cerevisiae* and *Candida lipolytica,* and factor 2 is the fermentation time of 2, 4, and 8 days and repeated four times, so there are 24 treatment units. The data was analyzed using analysis of variance and continued with the Least Significant Difference test. The research results showed that TPC produced an average value of 17.75×10^9 CFU/mL 37.75×10^9 CFU/mL, pH 4.69 6.51, temperature 26.775 30.925 °C, water content 32.51% 48.74%. The yeast type *S. cerevisiae,* with a fermentation time of 4 days, produces the best water content, while *C. lipolytica* with a fermentation time of 4 days produces the best TPC and temperature. Using *C. lipolytica* with a fermentation time of 8 days produces the best pH.

Keywords: Local soybean; Fermentation; *Saccharomyces cerevisiae*; *Candida lipolytica;* Yeast

1. Introduction

Chicken is one of the most economical sources of animal protein. The chicken farming business is highly dependent on the feed provided. Feed plays an essential role in increasing livestock productivity and business success. Feed costs in the livestock business range from 60 to 70% of the total production cost [1]. Therefore, it is essential to evaluate quality feed ingredients that are easy to process and do not require high production costs. Nutrients that must be included in the formulation of feed rations are carbohydrates, proteins, fats, minerals, and vitamins [2]. One of the essential components in feed is protein. Protein is beneficial for the growth of farm animals. A commonly used source of protein for animal feed is soybean meal. Soybean meal, widely known as *Soy soybean meal* for animal feed, is usually obtained from imports. This results in feed prices increasing from year to year. Therefore, it is necessary to have alternative feed ingredients to replace soybean meal.

Local soybeans are an alternative feed ingredient that can be used. Dried soybeans contain nutrients: 34% protein, 19% oil, 34% carbohydrates, 17% fiber, 5% minerals, and several other components, including vitamins and *isoflavones* [3]. According to [4], in the manufacture of soybean-based tofu, the quality of local soybeans is superior to imported soybeans. However, the use of local soybeans in Indonesia is still lacking. This is because the people of Indonesia are still dependent on imported soybeans, whose prices fluctuate. This is quite interesting because soybean production in Indonesia is quite large. According to estimates from the Directorate of Miscellaneous Beans and Tubers, soybean production in Indonesia in 2020 was 290.78 thousand tons of dry seeds, and in 2021 it decreased to around 212.86 thousand tons. The way to improve the quality of soybeans is by fermentation techniques.

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Fermentation is a chemical change in food caused by enzymes; enzymes that play a role can be produced by microorganisms or already in the food. Yeast fermentation can increase the nutritional and functional properties of food and reduce anti-nutrient substances. Fermentation is a process of biochemical change of food ingredients involving the activity of microorganisms and metabolites of the activity of the enzymes of these microorganisms [5]. Fermentation can reduce the anti-nutrient levels of a feed ingredient [6]. Fermentation can improve nutritional quality, reduce or eliminate the negative influence of certain feed ingredients, increase digestibility value, add taste and aroma, and increase the content of vitamins and minerals [7]. In this study, the types of yeast used were *S. cerevisiae* and *C. lipolytica*.

S. cerevisiae is a yeast that is often found in the processing of food products and the fermentation of animal feed. *S. cerevisiae* is used because it has a high fermentation rate and resistance to environmental pressure, including tolerance to alcohol products[8]. According to [9], *s. cerevisiae* can produce lipase enzyme if grown on a substrate containing fat; this decrease in fat content can be caused by the activity of the lipase enzyme produced by these microbes to remodel the fat content of the substrate as an energy source for its growth. *C. lipolytica* is a non-conventional yeast, not pathogenic, and generally considered a safe yeast [10]. The name of the species "*lipolytica*" comes from its ability to hydrolyze fat. This type of yeast can produce lipase enzymes that hydrolyze fats. *C. lipolytica* can produce intracellular and extracellular enzymes such as lipase [11]. Continued that in their growth, this type of yeast is influenced by the incubation temperature, pH of the culture medium, and the inoculum.

The success factor of fermentation can be seen in the growth of TPC, which is also commonly known as TPC. TPC is a microbiological method used to calculate the density of microbial populations in a sample. Factors that affect the number of Yeast colonies are the inoculum size, the growth medium, and the incubation time. pH is one of the determining factors of successful fermentation. The function of pH measurement is to determine the level of alkalinity or acidity possessed by a solution, pH provides an essential role in fermentation, affecting enzyme activity, microbial growth, and product spectrum. As low pH becomes more acidic, the rate of fermentation increases; this is because the organisms that allow glucose to ferment have adapted to a low pH environment. In the fermentation process, the pH usually decreases to become more acidic and then increases after some time; this is due to microorganisms consuming nutrients and producing organic acids that are released into the medium, so the pH decreases. Temperature measurement is done to monitor the fermentation process going well, in the fermentation process, temperature is very important for the growth and activity of microorganisms involved. The optimum temperature depends on the type of microorganism and the type of substrate used in the fermentation process. Temperature has a significant impact on the production of enzymes by yeast during fermentation.

The highest reaction speed occurs at the optimum temperature. The optimum temperature is when the enzyme's activity reaches its peak. Temperatures that are too high, which are far from the optimal temperature of an enzyme, can lead to enzyme denaturation. Moisture content measurement is also carried out, and moisture content is one of the good indicators of whether a feed ingredient is suitable to be one of the ingredients in the feed formulation. Moisture content plays an essential role in microbial metabolism. Water availability affects the production of enzymes, growth, and diffusion of microorganisms. The moisture content of the final fermentation product can also be reduced or increased. The high water content occurs due to respiration, which results in the decomposition of many nutrients, resulting in a significant decrease in the dry matter content as the fermentation time progresses.

In a study by [12], fermentation of coffee husks (*Coffee arabica*) using *S. cerevisiae* for two days produced the best parameter values. In a study [13], fermentation of Arabica coffee powder (*Coffe arabica* L) using *S. cerevisiae* yeast, it was found that samples with an additional concentration of *S. cerevisiae* 4% had the lowest pH value from Arabica ground coffee brewing. According to [14], a suitable pH value for anaerobic fermented feed is 3.2 4.2. In research [15], fermentation using *C. lipolytica yeast* to increase biodiesel production can increase the moisture content during the fermentation process. The value of the optimum moisture content for the result of the fermentation process ranges from 22.4 75%, depending on the type of product, substrate, and selection of organisms [16]. In a study [17], the yeast *C. lipolytica* can grow optimally at 28 35 °C. TPC is a method commonly used in calculating the total number of microorganisms (mold, yeast, bacteria) in a material [18].

2. Materials and methods

2.1. Research Materials

The feed ingredients used in this study are local soybeans, as much as 20 kg. This local soybean is obtained from the Cikuda Hegarmanah Market, Jatinangor, Sumedang Regency, West Java-Indonesia. The inoculum used for the fermentation of local soybeans is *S. cerevisiae* and *C. Lipolytica.*

2.2. Research Methodology

The experiment used the Design Complete Random nested patterns with two factors. Factor 1 is the use of yeast (*S. cerevisiae* and *C. lipolytica*), and factor 2 is the fermentation time (2, 4, and 8) days and repeated four times, resulting in the following information: Y1T1 = *S. cerevisiae,* fermentation time length two days; Y1T2 = *S. cerevisiae,* fermentation time length four days; Y1T3 = *S. cerevisiae,* fermentation time length eight days; Y2T1 = *C. lipolytica,* fermentation time length two days; Y2T2 = *C. lipolytica,* fermentation time length four days; Y2T3 = *C. lipolytica,* fermentation time length eight days; each repeated four times. So, there are 24 treatment units. The experimental data obtained were then statistically analyzed using Variety Analysis.

2.3. Observed variables

2.3.1. TPC (Total Plate Count)

- Make the medium using agar solution and sterilized petri-dishes
- Dilute the inoculum yeast solution (10^{-7}, 10^{-8}, 10^{-9}) using HCl and take 1 ml
- Plant each dilution in a petri dish filled with agar
- Let stand for eight days and count TPC on days 2, 4, and 8

The principle of the TPC method is to grow cells of microorganisms that are still alive on the agar medium so that microorganisms will multiply and form colonies that can be seen directly and counted by the eye without using a microscope.

2.3.2. pH

- Set of temperature regulators pH meter at measured temperature.
- Turn on the pH meter and steady it (15 30 minutes).
- Rinse the electrodes with sample aliquots or aquaades (if using aquades, dry the electrodes with tissue paper).
- Dip the electrode of the sample solution and set the pH meter.
- Allow the dip electrode to dip until a stable reading is obtained.
- Record the pH of the sample.

2.3.3. Temperature

- Prepare fermented soybeans
- Prepare a mercury thermometer
- Plug a mercury thermometer into fermented soybeans
- Record the temperature number displayed on the thermometer
- Do three repetitions at three different anchoring points, then calculate the average

2.3.4. Moisture

- Dry the cups in the oven for 1 hour at 100 105 °C
- Then, put it in the desiccator to cool for 15 minutes and then weigh it using an analytical scale (A)
- After knowing the zero weight of the analytical scale, then insert a sample of ±2 grams (B)
- Then put the cup containing the sample in the oven, and dry it at 100 105 °C for 6 hours, then weigh (C)

$$\text{Moisture (\%)} = \frac{B-C}{B-A} \text{ x } 100\ \%$$

3. Results and Discussion

3.1. Total Plate Count (TPC) Analysis Results

The calculation of TPC is carried out to measure the success of fermentation by calculating the density of the microbial population in Table 1.

Table 1 Effect of Fermentation on Local Soybean to TPC

Treatment	Deuteronomy				Average	Average
	1	2	3	4		
	.....×10⁹ CFU/ml.....					
Y1T1	36	37	34	35	35	27
Y1T2	27	22	28	30	26	
Y1T3	22	19	18	20	19	
Y2T1	23	20	28	19	22	26
Y2T2	37	38	37	39	37	
Y2T3	15	21	20	15	17	

Information: Y1T1 = *S. cerevisiae,* fermentation time length two days; Y1T2 = *S. cerevisiae,* fermentation time length four days; Y1T3 = *S. cerevisiae,* fermentation time length eight days; Y2T1 = *C. lipolytica,* fermentation time length two days; Y2T2 = *C. lipolytica,* fermentation time length four days; Y2T3 = *C. lipolytica,* fermentation time length eight days.

The TPC of fermentation increased on the 2nd and 4th days, then decreased on the 8th day with an average TPC value of 17.75×10^9 CFU/mL 37.75×10^9 CFU/mL. The type of yeast had no significant effect ($P>0.05$), but the highest TPC value was obtained in *C. lipolytica* with a fermentation period of 4 days (Y2T2) with a value of 37.75×10^9 CFU/mL. Meanwhile, yeast nested at the time had a real influence ($P<0.05$) on local soybean fermentation. Therefore, further tests were carried out to determine the difference in the average TPC (*C. lipolytica*) between fermentation times using the Smallest Real Difference Test in Table 2.

Table 2 The Smallest Real Difference Test of the Effect of Fermentation Time on TPC *C. lipolytica*

Treatment	Average	Average + BNT	Symbol
Y2T3	17.75	21.76	a
Y2T1	22.50	26.51	b
Y2T2	37.75	41.76	c

The results of the minor real difference test showed a difference between the treatments for TPC. Local fermentation of soybeans with *C. lipolytica* with a duration of 4 days (Y2T2) was significantly different ($P<0.05$) from the duration of 2 days (Y2T1) and the duration of 8 days (Y2T3). In local soybean fermentation using *C. lipolytica* on day 4 (Y2T2), it showed the highest average TPC compared to day two and day 8. The highest TPC value in fermentation using *C. lipolytica* on day 4 was 37.75×10^9 CFU/mL. The total increase in yeast fermentation on day 4 is due to the microbes being at the growth peak or stationary. In this phase, the growth speed is influenced by the medium where it grows such as pH, nutrient content, and environmental conditions, including temperature and air humidity [19]. This corresponds to the temperature produced in local soybean fermentation with a duration of 4 days (Y2T2) resulting in the highest temperature.

On day two, it can be seen that the growth of yeast is not as much as the growth on day 4. This is because, at the beginning of fermentation, microbes are still in the adaptation phase to adjust to the surrounding environmental conditions. The adaptation phase is characterized by an increase in macromolecular components, metabolic activity, and susceptibility to chemical substances and physical factors; this phase is characterized by the absence of an increase in cell mass or the number of cells [20]. The length of this adaptation phase can be affected by the number of inoculated cells, the appropriate physiological and morphological conditions, and the required cultivation medium [21].

On the 8th day, the TPC value decreased. This is because there is a phase of death in microbes. At this time, the number of dead cells is more than the number of living cells; this is because the nutrients and energy in the cells are depleted. The logarithmic form of the death phase is a straight-line decrease depicted by the number of living cells over time and the number of living microbes decreasing [21]. In the phase of microbial death, when nutrients are exhausted, the microbial population will decrease in number [21]. The death speed depends on nutrient conditions, the environment, and the type of microbe.

Fermentation kinetics studies microbial proliferation, which is indicated by increased biomass concentration due to substrate consumption. Based on Table 2, it can be seen that the highest number of microbes is found in the fermentation *of C. lipolytica* on day 4, which is 37.75×10^9 CFU/mL. After that, it decreases again on the next fermentation day.

3.2. Temperature Analysis Results

The optimum temperature for the type of microorganism and substrate used can vary. The optimum temperature is when the enzyme activity reaches its peak. In Table 3, the results of temperature analysis of local soybean fermentation can be seen.

Table 3 Effect of Fermentation on Local Soybean Temperature

Treatment	T0	Deuteronomy				Average	Average
		1	2	3	4		
	.....°C.....						
Y1T1	28.0	27.5	27.3	26.3	26.0	26.7	29.2
Y1T2		31.3	30.3	30.3	30.2	30.5	
Y1T3		30.7	30.7	30.0	30.3	30.4	
Y2T1		27.0	28.8	28.7	27.0	27.8	29.9
Y2T2		32.0	30.7	30.5	30.5	30.9	
Y2T3		32.0	31.3	29.7	30.7	30.9	

Information: Y1T1 = *S. cerevisiae,* fermentation time length two days; Y1T2 = *S. cerevisiae,* fermentation time length four days; Y1T3 = *S. cerevisiae,* fermentation time length eight days; Y2T1 = *C. lipolytica,* fermentation time length two days; Y2T2 = *C. lipolytica,* fermentation time length four days; Y2T3 = *C. lipolytica,* fermentation time length eight days.

The temperature of the fermented product increased on the 4th day but decreased on the 2nd day with an average temperature value of 26.77 30.92°C. The type of yeast had no significant effect ($P>0.05$), but the highest temperature value was obtained in *C. lipolytica,* with an average value of 29.9 °C. Meanwhile, yeast nested at the time had a real influence ($P<0.05$) on local soybean fermentation. Therefore, further tests were carried out to determine the difference in the average temperature (*C. lipolytica*) between fermentation times using the Smallest Real Difference Test, presented in Table 4.

Table 4 The Smallest Real Difference Test The Effect of Fermentation Duration on Temperature

Treatment	Average	Average + BNT	Symbol
Y2T1	27.87	29.00	a
Y2T2	30.92	32.05	b
Y2T3	30.92	32.05	b

The results of the Smallest Real Difference Test (Table 4) showed that there was a difference in the treatment of temperature. The effect of using *C. lipolytica* with a real 2-day fermentation time (Y2T1) ($P<0.05$) was lower than that of *C. lipolytica* with a fermentation time of 4 days (Y2T2), and the use of *C. lipolytica* with a fermentation time of 8 days (Y2T3). Using *C. lipolytica* with a fermentation period of 4 days (Y2T2) and eight days (Y2T2) resulted in the highest temperature of 30.92 °C.

The local soybean temperature before fermentation is 28 °C (Table 3), then the temperature increases after the fermentation process with an average of 26.77 °C 30.92 °C (Table 3). The increase in temperature is caused by the yeast activity that can produce heat during fermentation. The best treatment that delivers the highest temperature is what is the treatment of (Y2T2) *C. lipolytica* with a fermentation time of 4 days and (Y2T3) *C. lipolytica* with a fermentation time of 8 days, which is 30.92 °C. If the fermentation time of local soybeans is less than four days, the resulting temperature is lower, which means that the activity of the yeast is not optimal. Microbial activity from fermentation can produce alcohol acids and release heat due to exothermic reactions [22]. The growth of microorganisms will increase along with

the increase in environmental temperature, increasing the rate of microorganism populations [23]. This is by the highest population increase in the calculation of TPC with (Y2T2) treatment of *C. lipolytica* with a fermentation period of 4 days, which is 37.75×10^9 CFU/mL.

The growth of microorganisms is greatly influenced by the ambient temperature of the environment. Temperature plays an active role in enzyme activity or metabolic processes in microorganisms, where microorganisms have the optimal working temperature [24]. According to [25], The optimal temperature for the growth of yeast *C. lipolytica* is from 20 °C – 40 °C, and can grow at a maximum of 29 °C. The heat produced in the fermentation process comes from microbes that carry out metabolic activities to convert glucose into alcohol and CO_2 gas, accompanied by the release of heat energy [13]. The best treatment was obtained with the use of *C. lipolytica* with a fermentation period of 4 days (Y2T2) because the temperature in the treatment was still within the optimal growth temperature and was directly proportional to the TPC value produced, whereas the use of *C. lipolytica* with a fermentation period of 4 days (Y2T2) produced the highest TPC

3.3. pH Analysis Results

pH measurement is carried out to determine the degree of acidity and alkalinity of a material. The rate of enzyme activity is determined by pH and temperature. Each type of enzyme has an optimal pH for its activity. In Table 5, the results of the pH analysis of local soybean fermentation can be seen.

Table 5 Effect of Fermentation on Local Soybean pH

Treatment	P0	Deuteronomy				Average	Average
		1	2	3	4		
	.....pH.....						
Y1T1	6.00	6.52	6.76	6.29	6.45	6.51	5.85
Y1T2		6.03	6.19	6.09	6.56	6.22	
Y1T3		4.64	5.35	4.53	4.82	4.84	
Y2T1		6.43	5.90	6.26	6.85	6.36	5.81
Y2T2		6.55	6.91	5.53	6.58	6.39	
Y2T3		4.84	4.62	4.10	5.20	4.69	

Information: Y1T1 = *S. cerevisiae,* fermentation time length two days; Y1T2 = *S. cerevisiae,* fermentation time length four days; Y1T3 = *S. cerevisiae,* fermentation time length eight days; Y2T1 = *C. lipolytica,* fermentation time length two days; Y2T2 = *C. lipolytica,* fermentation time length four days; Y2T3 = *C. lipolytica,* fermentation time length eight days.

The pH of the fermentation results increased on the 2nd and 4th days, then decreased on the 8th day with an average pH value of 4.69 6.51. Based on the ANOVA, the type of yeast had no discernible effect ($P>0.05$), but the lowest pH value was obtained in *C. lipolytica* with an average value of 5.81. Meanwhile, yeast nested at the time had a real influence ($P<0.05$) on local soybean fermentation. Therefore, further tests were carried out to determine the difference in the average pH (*C. lipolytica*) between fermentation times using the Smallest Real Difference Test, presented in Table 6.

Table 6 The Smallest Real Difference Test of the Effect of Fermentation Time on the pH of *C. lipolytica*

Treatment	Average	Average + BNT	Symbol
N2W3	4,69	5,28	a
N2W1	6,36	6,95	b
N2W2	6,39	6,98	b

The fermentation of *C. lipolithcya* with a duration of 4 days (Y2T2) was significantly different ($P<0.05$) compared to the fermentation of *C. lipolithcya* with a duration of 8 days (Y2T3) and the fermentation of *C. lipolithcya* with a duration of 2 days (Y2T1). The pH value of local soybeans before fermentation is 6.00 (Table 5). After fermentation, the pH of local soybeans decreased by 19 21% on the 8th day and increased on the 2nd and 4th days with an average pH of 4.69 6.51 (Table 5). When the fermentation period is less than eight days, the pH value has not dropped optimally, meaning that

the activity of the yeast has not been maximized. The decrease in pH on day eight is caused by the activity of yeast that reshapes the substrate so that more organic acids are produced, and the pH will also decrease [26]. The decrease in pH can be influenced by several factors, namely the type of media, temperature, culture conditions, and the presence of salt [1].

The pH drop factor can be caused by the temperature during the fermentation process. As the fermentation temperature increases, CO_2 gas production will increase. The increased production of CO_2 gas will affect the pH value during fermentation because an acidic gas is formed from the CO_2 that is dissolved more and more. The higher the fermentation temperature, the lower the pH value. This is evidenced by the decrease in pH value, which is most abundant in local soybean fermentation using *C. lipolytica* yeast with a fermentation period of 8 days (Y2T3), producing the highest temperature of 30.92°C compared to other treatments. Therefore, it can be said that the pH value can also be affected by the production of CO_2 gas [27].

3.4. Moisture Content Analysis Results

Moisture content is essential in the fermentation process, especially in microbial metabolism. Water availability can affect enzyme production, growth, and diffusion of microorganisms. In the fermentation process, the water content value can decrease and increase.

Table 7 Effect of Fermentation on Local Soybean Moisture Content

Treatment	T0	Deuteronomy				Average	Average
		1	2	3	4		
	.....%.....						
Y1T1	9.25	29.13	40.78	29.13	31.00	32.51	40.03
Y1T2		43.56	58.86	46.76	45.81	48.74	
Y1T3		32.73	55.20	36.97	30.49	38.84	
Y2T1		35.92	35.92	31.37	37.86	35.26	36.72
Y2T2		46.33	38.10	37.40	36.22	39.51	
Y2T3		34.48	39.53	25.84	41.77	35.40	

Information: Y1T1 = *S. cerevisiae,* fermentation time length two days; Y1T2 = *S. cerevisiae,* fermentation time length four days; Y1T3 = *S. cerevisiae,* fermentation time length eight days; Y2T1 = *C. lipolytica,* fermentation time length two days; Y2T2 = *C. lipolytica,* fermentation time length four days; Y2T3 = *C. lipolytica,* fermentation time length eight days.

The lowest moisture content was achieved on day 2, with an average moisture content of 32.51% 48.74%. The type of yeast had no apparent effect ($P>0.05$), but the highest moisture content value was obtained in *S. cerevisiae,* with an average value of 40.03%. Meanwhile, yeast nested at the time had a real influence ($P<0.05$) on local soybean fermentation. Therefore, further tests were carried out to determine the difference in the average moisture content (*S. cerevisiae*) between fermentation times using the Smallest Real Difference Test in Table 8.

Table 8 The Smallest Real Difference Test of the Effect of Fermentation Time on the Moisture Content of *S. cerevisiae*

Treatment	Average	Average + BNT	Symbol
N1W1	32.51	42.72	a
N1W3	38.84	49.06	a
N1W2	48.74	58.96	b

The results of the Smallest Real Difference Test (Table 8) showed a difference between the treatments for water content. The fermentation *of S. cerevisiae* with a duration of 4 days (Y2T2) was significantly different ($P<0.05$) from the fermentation *of S. cerevisiae* with a duration of 8 days (Y2T3) and fermentation *of S. cerevisiae* with a duration of 2 days (Y2T1).

The moisture content of local soybeans before fermentation is 9.25% (Table 7). After fermentation, the moisture content of local soybeans increased by 251 427%. The lowest moisture content in using *S. Cerevisiae* was found on the 2nd day of fermentation, increased on the 4th day, and then decreased on the 8th day. The increase in moisture content is caused by yeast activity that can produce H_2O. The increase in moisture content in the fermentation process is caused by the activity of microbes during the fermentation process, which can increase the moisture content [28]. The fermentation process that produces water from metabolism is an indicator of the sustainability of the fermentation process. The increased moisture content can also be caused by yeast that utilizes carbohydrates as a source of energy to grow. Sugar results from the reshuffle of carbohydrates that can be used as energy with by-products in metabolites, acids, CO_2, and water [29]. On the 8th day of fermentation using *S. cerevisiae* (Y2T3), the moisture content of local soybeans decreased because the yeast activity had decreased, so there was no reshuffle of yeast compounds that could increase the moisture content. This is to the TPC results produced on day 8. The decrease in moisture content can also be caused by evaporation during the fermentation process. The longer the fermentation causes more moisture content to be released, the texture of the material becomes soft and porous [30]. Changes in texture and pores will make it easier to evaporate from water; from this state, the moisture content will decrease.

4. Conclusion

The use of yeast type and the length of fermentation time had a noticeable effect on the TPC, pH, temperature and moisture content of the study. Using *C. lipolytica* with a fermentation period of 4 days (Y2T2) produced the best TPC value and temperature. Using *C. lipolytica* with a fermentation period of 8 days (Y2T3) yielded the best pH value. Using *S. cerevisiae* with a fermentation period of 4 days (Y1T2) produced the best moisture content value.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest is to be disclosed

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