Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 4587 - 4595

Received 25 April 2021: Accepted 08 May 2021.

Nutritional Profile of Pineapple Skin Fermented by Yeast culture (*Saccharomyces spp.*) and the Ability to Inhibit the Growth of *Escherichia coli* bacteria

BULKAINI[1], I Gusti Nyoman Gde BIDURA[2*], I Ketut SUMADI[2], dan I Nyoman Tirta ARIANA[2]

[1] Faculty of Animal Science, University of Mataram, Lombok, Indonesia

[2] Faculty of Animal Science, Udayana University, Denpasar-Bali, Indonesia

*Correspondensi: bidura_unud@yahoo.com

ABSTRACT

The study was conducted to see changes in nutrients in pineapple skin fermented (PSF) with tape yeast (*Saccharomyces spp*.) and their inhibitory power against the growth of *E. coli* bacteria. This study used a one-way completely randomized design with 4 treatments and 5 replications. The treatments for observing the nutritional profile (crude protein/CP and crude fiber/CF) of PSF and the number of *Saccharomyces spp* bacteria colonies were as follows: P0 = PSF without using tape yeast; P1 = PSF using 0.5% yeast tape; and P2= PSF using 1% yeast tape; P3 = PSF using 1.5% yeast tape, respectively. For the *E. coli* anti-bacterial test, namely P0 = negative control using distilled water, P1 = positive control using the antibiotic ciprofloxacin, P2 = pineapple skin extract (PSE) with a concentration of 75%; P3 = PSE with a concentration of 85%; and P4 = PSE with a concentration of 100%, respectively. The results showed that PSF with 1% yeast tape significantly ($P<0.05$) increased CP content and significantly ($P<0.05$) decreased CF content in PSF. The percentage of use of tape yeast in fermentation had a significant effect ($P<0.05$) on the number of *Saccaharomyces spp* colonies. Inhibition test of PSE against the growth of *E. coli* bacteria, at a concentration of 70% and 85% it was classified as a weak category, while at a concentration of 100% it was classified as moderate category. It can be concluded that PSE has the ability to inhibit the growth of *E. coli*, and the use of 1% Yeast culture in fermentation of pineapple peels can increase CP content and reduce the CF content of pineapple peels.

Keywords: Saccharomyces spp. Protein, fiber, E. coli

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Introduction

Pineapple peel is a waste of pineapple fruit that is 25-35% of the weight of pineapple or 20-24% of the weight of pineapple without leaves[1]. The skin of pineapple fruit, has nutritional value consisting of 85.78% dry matter, 81.90% organic matter, 8.1% ash, 3.50% crude protein, 19.69% crude fiber, 3.49% crude fat, neutral digestible fiber (NDF) 57.27% and is a feed source of energy with a gross energy content of 4,481 kcal[2]. Fresh pineapple skin contains 91.35% water[3], and to extend its shelf life, it is necessary to carry out a fermentation process[4].

Fermentation is a process of chemical changes in an organic substrate through the activity of enzymes produced by microorganisms, so that it is able to convert complex macromolecules into simple molecules that cause feed to be digested easily by poultry and does not produce toxic chemical compounds[5,6,7,8].

Fermentation can improve the nutritional profile of a feed ingredient, such as protein content, crude fiber, crude fat and other nutritional content[9]. Furthermore, it was said that fermented cacao pods with rumen fluid inoculum increased the nutritional profile (protein) from 9.62% to 9.81%, and when using the "Bioplus" inoculum, the protein increased to 10.21%. Fermentation of the cocoa pods caused the protein to increase from 7.38% to 9.61%[10].

Yeast tape (*Saccharomyces cereviceae*) is a starter for the manufacture of rice tape or cassava tape containing microorganisms such as Chlamydomucor oryzae, Rhizopus oryzae, Mucor sp., Candida sp., *Saccharomyces cerevisiae*, and *Saccharomyces verdomanii* [11,12]. *Saccharomyces cereviceae* in poultry can reduce *Escherichia coli* (*E.coli*) and increase body weight[13]. Furthermore, it was reported that the advantage of using Saccharomyces cereviceae as a probiotic is that it does not kill the digestive tract microbes, but increases the number of beneficial microbes. Unlike the case with antibiotics that can kill

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 4587 - 4595

Received 25 April 2021: Accepted 08 May 2021.

pathogenic microbes, as well as beneficial microbes in the digestive tract of poultry, and have a resistance effect.

In the fermentation process with tape yeast, besides improving the nutritional profile of feed ingredients, it can also increase the number of Saccharomyces spp colonies[14]. The increase in the number of *Saccharomyces spp* colonies depends on the concentration of tape yeast used, namely the higher the concentration of tape yeast used, the greater the number of Saccharomyces spp colonies[11].

The antibacterial activity of pineapple peel extract against several types of bacteria is different, depending on the type and concentration of the solvent used[15,16]. The inhibition zone of pineapple peel extract with acetone solvent at a concentration of 50 mg /ml against the growth of *Salmonella typhii* bacteria is 18 mm; *Pseudomonas aeruginosa* by 15 mm; 14 mm *Streptococcus pyrogenes*; and by 13 mm against the *Proteus vulgaris* bacteria. Pineapple peel extract with ethanol solvent has an inhibition zone against *Eschericia coli* (*E.coli*) bacteria of 16.5 mm[17]. Based on the description above, a study aimed to see changes in nutrients in pineapple skin fermented with tape yeast (*Saccharomyces spp*), the number of *Saccharomyces spp* colonies and their inhibitory power against the growth of *E. coli* bacteria.

Materials and Methods

Research material and experimental design

The materials used in this study were pineapple peel, yeast tape, molasses and chemicals used for proximate analysis of the nutritional content of fermented pineapple peels. Chemicals for the E. coli antibacterial test: Eosin Methylene Blue Agar (EMB) media, Muller Hinton Agar (MHA) media, E. coli bacteria cultures, cotton, distilled water, disc paper, filter paper, methylated spirits, sterile distilled water, 96% ethanol solution, ciprofloxazin antibiotic, 70% alcohol and a standard solution of 0.5 Mc Farland. The chemicals for calculating the number of colonies of *Saccharomyces spp*. were: Potato Dectose Agar (PDA) medium and 0.9% NaCl physiological solution.

This study used a one-way completely randomized design with 4 treatments and 5 replications. The treatments for observing the nutritional profile (CP and CF) of PSF and the number of *Saccharomyces spp*. bacteria colonies were as follows: P0 = PSF without using tape yeast; P1 = PSF using 0.5% yeast tape; and P2 = PSF using 1% yeast tape; P3 = PSF using 1.5% yeast tape. For the E. coli anti-bacterial test, namely P0 = negative control using distilled water, P1 = positive control using the antibiotic ciprofloxacin, P2 = PSE with a concentration of 75%; P3 = PSE with a concentration of 85%; and P4 = PSE with a concentration of 100%, respectively.

Pineapple Skin Fermentation Process

Pineapple peel fermentation is carried out with the following procedures: (1) First the pineapple skin is cleaned, then chopped and dried in the sun, then milled, so that it becomes flour[18] ; (2) The pineapple skin flour is then steamed for 30 minutes so that it is sterile from various kinds of microbes, such as fungi and other bacteria that can interfere with the fermentation process[11,19] ; (3) After chilling, then added tape yeast with different percentages according to the treatment; (4) Provide molasses solution with a concentration of 25% (200 ml molasses: 800 ml sterile water); (5) Each treatment was sprayed with a solution of molasses that had been prepared beforehand, while stirring evenly until a fist of pineapple peel flour was formed; (6) The results of spraying the pineapple peel flour with the molasses solution are then put into a loose-closed black bucket to obtain a facultative anaerobic condition, then incubated at room temperature and not exposed to sunlight for 4-6 days; (7) After the incubation period was complete, the fermentation product of each treatment is partially taken for the purposes of proximate analysis and testing of the number of Saccharomyces spp.

The process of making pineapple peel extract.

The work steps of making PSE begin by washing the pineapple skin until it was clean, then the pineapple skin that has been prepared was cut into small pieces and oven-dried at 50ºC. After drying, then mashed in a blender and weighed 150 grams using an analytical balance. Furthermore, the sample was extracted by maceration, which was immersed in 600 ml of ethanol for 48 hours in a place that was

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 4587 - 4595

Received 25 April 2021: Accepted 08 May 2021.

not exposed to direct sunlight with occasional stirring. This maceration process was carried out once. Furthermore, the results of the maceration are filtered to separate the filtrate and the residue. The resulting filtrate was evaporated using a rotary evaporator to separate the solvent, so that a thick pineapple peel extract was obtained, approximately 24.6 g.

The variables observed were: nutritional profile of fermented pineapple peel, namely: CP and CF content, obtained by doing proximate analysis[20]. Number of Saccharomyces spp. colonies: Calculation of the number of colonies of *Saccharomyces spp.* was done using a Standard Plate Count (SPC) by means of a Petridis dish containing yeast colonies, calculated using a colony counter[21]. The inhibition of PSE against the growth of E. coli bacteria was carried out using the diffusion method[15].

Statistic analysis

The research results were analyzed using one-way-anova analysis and continued with the Duncan multiple range test at a 95% confidence level using the SPSS version 16 program.

Results

Nutritional profile of fermented pineapple peel

The nutritional profile of PSF using tape yeast (*Saccharomyces spp*.) is presented in Table 1. The results of the one way ANOVA analysis showed that the percentage of use of tape yeast in the fermentation of pineapple peels had a significant effect ($P<0.05$) on the nutritional profile of pineapple peels (Table 1). The Duncan test results showed that the use of tap yeast at the 1% level provided a higher crude protein content (6.614%) and significantly different ($P<0.05$) with P0 (5.635%) and P1 (6.246%). The crude protein content in each treatment of pineapple skin fermented using tape yeast increased, namely at P1 to 6.246%, followed by P2 by 6.614% and decreased in P3 to 6.499%. The results of this study are in line with the results of research by[22], who found that rice bran fermented with yeast containing Saccharomyces spp. can increase its protein content, from 10.93% to 13.27%. Furthermore, it was also reported that the semi-solid fermentation process could increase the crude protein feed. The increase in protein is due to the process of changing inorganic N (nitrogen) in the form of urea and ammonium sulfate (SA) by yeast into organic N (protein).

Table 1. Nutritional profile of pineapple peel fermented with Tape yeast (*Saccharomyces spp*).

Nutrient	**Percentage of Tape Yeast (%)**			
	P0 (0%)	**P1 (0.5%)**	**P2 (1.0 %)**	**P3 (1.5%)**
Crude protein (%)	5.635 ± 0.174^{a}	6.246 ± 0.023^{b}	6.614 ± 0.021^{c}	6.499 ± 0.006^{c}
Crude fibre (%)	11.740 ± 0.236^{a}	11.390 ± 0.198^{b}	10.786 ± 0.114^{c}	10.567 ± 0.105^{c}

Note: Different superscripts on the same line indicate a significantly difference ($P<0.05$).

Pineapple peel fermented with 2% *Aspergillus niger* for 4 days, the CP content increased from 6.75% to 9.55%[23]. The use of 5% *Lactobacillus* as a fermentation inoculum can increase the CP content of fermented pineapple peels from 1.21% to 1.67%[24]. The increase in protein is thought to be due to the addition of protein contributed by microbial cells as a result of its growth, which results in single cell protein (SCP) products or cell biomass containing about 40-65% protein[25]. The same thing was reported by The increase in crude protein content of fermented pineapple peels is caused by the microbes used in the fermentation process, which continue to experience growth and will reach their peak at a certain time depending on the type of microbe[26]. Microbial growth in the form of molds will produce more biomass in fermentation products. Affirmed that in Yeast Tape contained about 8x107 cells/g to 3x108 cells/g mold; 3x106 to 3x107 cells/g Yeast; and 103 cells / g of bacteria[27].

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Received 25 April 2021: Accepted 08 May 2021.

Production of yeast *Saccharomyces spp* in fermented pineapple skin

The logarithm graph of the development of the number of *Saccharomyces spp* colonies on fermented pineapple skin using tape yeast is presented in Figure 1.

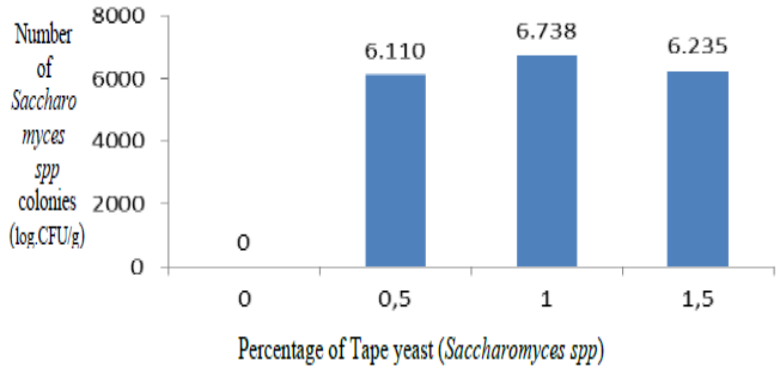

Figure 1. Graph of the development of the number of *Saccharomyces spp* colonies on fermented pineapple skin

Based on Figure 1 above, it can be seen that the percentage of tape yeast use has a significant effect (P<0.05) on the number of *Saccaharomyces spp*. The number of *Saccharomyces spp* colonies at P0 was significantly different (P<0.05) with treatment P1 (6.110±0.156 log CFU/g); P2 (6.738± 0.143log.CFU/g); and P3 (6.235 ± 0.096 log CFU / g). The number of *Saccharomyces spp* colonies at P1 and P3 was significantly (P <0.05) lower than in P2 (Figure 1).

At a concentration of 1% tape yeast can produce the highest *Saccharomyces spp*. colonies, namely: 6.738 ± 0.143 log.CFU/g, and a decrease in saccharomyces spp colonies on tape yeast administration by 1.5% to 6,235 ± 0.096 log.CFU/g. The increase in the colonies of *Saccharomyces spp*. to a level of 1% of tape yeast was probably due to the level of 1% of the microbial tape yeast producing Saccharomyces spp. colonies that were still active, while increasing the level of taper yeast giving to 1.5% caused the working microbes to become inactive. It is said that the addition of a media concentration to the fermentation process can lead to the death of bacteria colony-producing microbes[32].

Inhibition of fermented pineapple peel against *E. coli* bacteria.

Observation of inhibition is done by measuring the diameter of the zone of inhibition. The inhibition zone indicators used are: weak inhibitory power has a diameter of <5 mm; a diameter of 5-10 mm is classified as moderate; 10-20 mm in diameter is classified as having a strong inhibitory power; and a diameter >20 is classified as very strong[34]. The results of the antibacterial activity test of pineapple peel extract with various concentrations against *E. coli* can be seen in Figure 2.

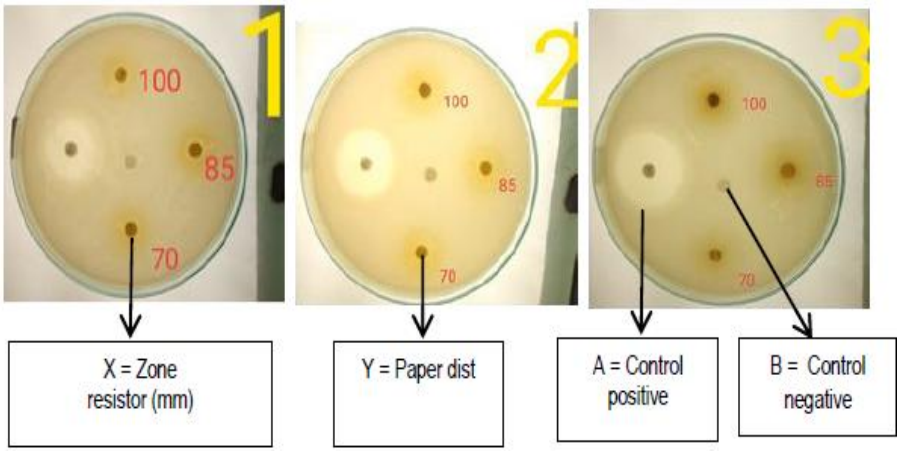

Figure 2. The results of the antibacterial activity test of pineapple peel extract (Number 70; 85; and 100 shows the concentration of pineapple peel extract).

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Figure 2 shows the value of the inhibition zone which is determined by the size of the diameter of the circle surrounding the paper dist. The greater the measurement results of the circle surrounding the paper dist, indicating that the pineapple peel extract has a strong inhibitory power against *E. coli* bacteria[15]. The factors that affect the size of the diameter surrounding the paper dist are largely determined by the number of secondary metabolite compounds, such as flavonoids, alkaloids, and saponins contained in an extract.

The average inhibition zone value of pineapple peel extract against *E. coli* bacteria is presented in Figure 3.

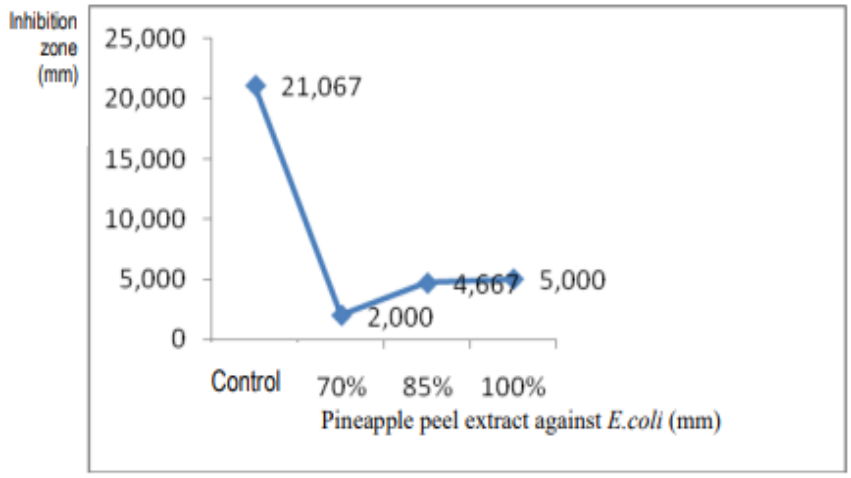

Figure 3. Graph of inhibition zone of pineapple peel extract against *E. coli* bacteria

Based on the results of the one-way ANOVA analysis and Figure 3 above, it can be seen that the concentration of pineapple peel extract has a significant effect ($P<0.05$) on the inhibition of growth of *E. coli* bacteria. Treatment with 70% concentration had 2 mm of inhibition and was significantly different ($P<0.05$) with treatment of 85% (4.667 mm) and treatment of 100% (5 mm). The value of inhibition between 85% and 100% treatments was not significantly different ($P>0.05$). From Figure 3, it can be seen that the inhibition power of pineapple peel extract against the growth of *E. coli* bacteria at a concentration of 70% and 85% is classified as weak with the inhibition power of 2 mm and 4.667 mm. Likewise, the concentration of 100%, it turns out that the inhibitory power is classified as moderate (5 mm). The results of this study were lower compared to the ethanol extract zone of pineapple skin against *E.Coli* bacteria by 16.5 mm which is classified as strong[17,34]. Pineapple peel acetone extract at a concentration of 50 mg/ml was able to inhibit the growth of the bacteria *Salmonella typhii*, *Pseudomonas aeruginosa*, *Streptococcus pyrogenes*, and *Proteus vulgaris* with inhibition zones respectively: 18 mm; 15 mm; 14 mm; and 13 mm. Meanwhile, the inhibitory power against the bacteria *Klebsiella pneumonia*, *Enterococcus faecalis* and *Staphylococcus aureus* forms an inhibition zone with a diameter of 12 mm[35].

Discussion

The CF content of pineapple skin fermented with tape yeast ranges from 10,567-11,740%. Crude fiber content of P2 was lower (10.786%) and significantly different ($P<0.05$) with P0 (11.740%) and P1 (11.390%). The crude fiber content of fermented pineapple peel at P2 (10.786%) was not significantly different ($P>0.05$) with P3 (10.567%). The decrease in crude fiber content in fermented pineapple peels at P2 and P3 indicates that the phytase enzymes found in tape yeast work actively in breaking down complex components or bonds into simple bonds. Yeast tape (*Saccharomyces spp*.) produces phytase enzymes that can release phosphorus bonds in phitin, so that complex bonds can be broken down into simpler bonds[28].

The CF content of fermented pineapple peel produced in this study ranged from: 10,567-11,740%, lower than the crude fiber content of fermented pineapple skin reported by[29], namely: 12.27% and[30] are:

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 4587 - 4595

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14.71%. The use of *Aspergillus niger* 2% with an incubation time of 4 days obtained a crude fiber content of pineapple peels of: 14.69%[23]. The use of 5% lactobacillus as the fermentation inoculum of pineapple peel was able to reduce crude fiber from 25.54% to 17.23% [24].

Fermentation of pineapple peels using various types of microbes can not always reduce the CF content of feed ingredients, on the contrary it can increase the CF content of agricultural waste depending on the length of fermentation time. The CF content of fermented feed was influenced by the growth of fungi (mycelium) in fungi, so that the longer the fermentation time, the dense mycelium growth and an increase in crude fiber content[6,31]. Furthermore, it was said that the longer the incubation time in the fermentation process, the higher the crude fiber content. This is due to the growth of molds which contribute to CF derived from the mycelium, so that the more cell mass the higher the fiber content.

Colony growth of Saccharomyces spp., on PDA (Potato Dextrose Agar) media culture of pineapple skin fermented with tape yeast is presented in Figure 4.

Figure 4 above shows that fermentation of pineapple peel using 1% yeast tape (P1) in PDA media culture resulted in the number of *Saccharomyces spp* colonies that are still active and read on a petridis plate of: 57 x 105 CFU/g; followed by the colony of *Saccharomyces spp*. on a petridis dish, giving tape yeast 1.5% (P3) of: 17.5 x 105 CFU/g, and giving tape yeast 0.5% (P1) of: 13.5 x 105 CFU/g, whereas in P0 treatment (without tape yeast) there were no *Saccharomyces spp*.

The results of the sample normality test using the One-Sample Shapiro-Wilk test, obtained results as listed in Table 2, where all treatments showed a p value> 0.05, which means that the data were normally distributed.

Table 2. Normality test results of fermented pineapple peel samples

Concentration	*Shapiro-Wilk*
Concentration 0.5%	P= 0.757
Concentration 1.0%	P= 0.606
Concentration 1.5%	P= 0.534

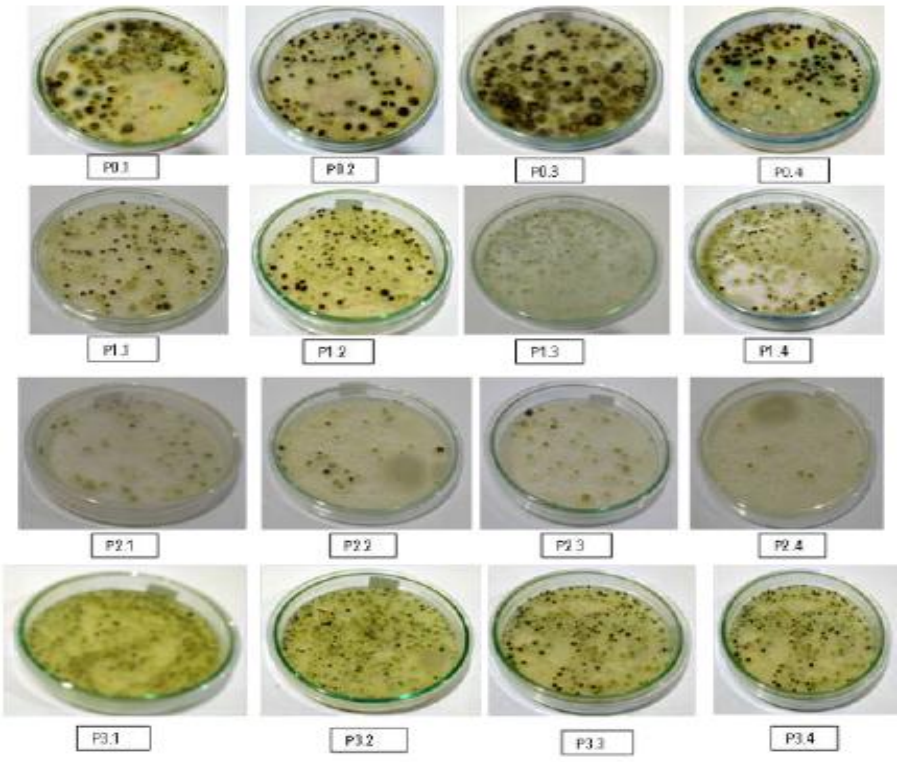

Figure 4. Colony growth of *Saccharomyces* spp. Fermented pineapple peel with tape yeast

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 4587 - 4595

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The results of the variance homogeneity test using Levene Statistics showed that the p value = 0.07 (Table 3), where the p value>0.05, which indicates that the variance is homogeneous, so it can be continued with the one-way ANOVA test.

Table 3. Variance homogeneity test

Levene Statistics	df1	df2	Sig.
3.057	3	12	0.070

The one-way ANOVA test results show that the significance value is smaller than 0.05, namely: 0.0001 ($p<0.05$). This means that there is a significant difference between groups of tape yeast concentration in growing the *Saccharomyces spp*. colonies found in fermented pineapple peels. Saccharomyces spp. It has more active life characteristics in conditions of high sugar concentration and an increasingly acidic fermentation pH (pH 3-4) when there is life competition with bacteria[33].

The low inhibitory power of the ethanol extract of pineapple peel against the growth of *E. coli* bacteria in all treatments in this study, because the solvent used in the mesarasi process is ethanol and is only done once, so that the content of phytochemical compounds contained in pineapple peel extract is not the maximum dissolved out as extracts, such as flavonoids, saponins, and tannins which ultimately affect the strength of the inhibitory power. The more flavonoids and saponins contained in the pineapple peel extract, the stronger the inhibition against bacteria will be[36,37]. Furthermore, it was explained that flavonoid and saponin compounds are secondary metabolite compounds that have the ability to be antibacterial, anti-inflammatory, and antioxidant. The diameter of the inhibition zone is highly dependent on the absorption of antibacterial substances into the agar plate and the sensitivity of bacteria to these antibacterial substance[38].

Conclusion

We can conclude that fermentation of pineapple peels using tape yeast (*Saccharomyces spp*.) can increase the protein content of fermented pineapple peels and reduce the crude fiber content. The inhibition of the ethanol extract from pineapple peel against the growth of *E. coli* bacteria is low.

Acknowledgements

The author would like to thank the Rector of the University of Mataram for his permission to study and all the staff of the Microbiology Laboratory, Faculty of Animal Husbandry, University of Mataram who have fully assisted the sample analysis.

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