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Effects of Glycerol and Chitosan Doses for Cassava Peels Organic Waste as Bioplastic Food Packaging and The Effects on Physical and Microbiological Food Quality

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Abstract— The long-term use of plastic for food packaging affects monomer migration. Bioplastics can be used as an alternative for food packaging because bioplastics can reduce risks of health. This research aimed to determine the effect of glycerol and chitosan doses for the utilization of cassava peel waste as bioplastics food packaging and its effect on physical and microbiological food quality. This research is experiment with the independent variable of glycerol and chitosan doses as (3 % weight: 3 ml, 5 % weight: 5 ml and 7 % weight: 7 ml) and the dependent variables are the physical and microbiological quality of food. Sample at this research is Getuk, with 24 samples Getuk (100 gr/sample). The result of the research is total plate count of control as 11500 CFU/g, treatment group 1 at 8783 CFU/g, treatment group 2 at 6716 CFU/g, and treatment group 3 at 4400 CFU/g. Analysis of statistics used ANOVA test, the result showed significant differences in doses of glycerol and chitosan for organic waste utilization of cassava peels as bioplastics food packaging to physical and microbiological food quality with a p-value as = 0,000. The conclusion of this research is the addition of 7 % weight chitosan and 7 ml glycerol showed the optimal treatment to reduce the total plate count of food (getuk) and the organoleptic test showed the food (getuk) packaging with bioplastics quality is better than regular plastic packaging. It's recommended to use bioplastics as food packaging because it's safer for health reasoning, further research is needed in determining the biodegradation time and other sources of microorganism.

Keywords: Glycerol, Chitosan, Bio plastics, Getuk (Traditional Javanese Wet Cake), and Organic waste of Cassava peels.

INTRODUCTION

The use of plastic in everyday life has become a common thing. Plastic is cheap and easy to use which makes it convenient for shopping bags and food packaging. In addition to these advantages, plastic also has a weakness, because the main raw material for making plastic derived from petroleum which is limited in number and is not renewable, moreover plastics can't be degraded quickly and naturally by the degradative microbes in the soil. This results in the accumulation of waste and causes environmental pollution and damage.[1]

The use of plastic packaging has become a necessity. Food packaging and food wrapping as food protection made from plastic that are used for a relatively long time will allow the migration or transfer of monomer substances from petroleum-based plastic materials into food. Monomer migration occurs because it is affected by food temperature and food pH. The higher the food temperature, the more of monomers that can migrate into the food.[1]

The Center for Chemical Research (LIPI) advises “avoid using plastic for food. Based on the results of research, food contaminated with plastic entering the body of experimental animals accumulatively can cause cancer, hormonal changes and cause ambigus genitalia. It is feared that this could also affect humans.[2]

One of the most commonly used plastic components, namely Bisphenol A (BPA), in 2014 the European Commission issued a BPA standard of 0.005 to 0.05 mg / kg / day and in 2011 a study in the United States showed that 90% of pregnant women were exposed to BPA, this is dangerous because it can cause cancer, impaired fetal brain development, and neurological disorders. [3]

The negative impacts of using plastics for health and the environment can be avoided by developing biodegradable plastics / bioplastics, namely plastics that can be naturally decomposed by microorganisms into environmentally friendly compounds. Various researches have been carried out in developed countries such as (Germany, France, Japan, Korea, the United States, Britain and Switzerland) with the aim of exploring various potentials of environmentally friendly polymer raw materials. In Germany, development to obtain biodegradable polymers using polyhydroxy butyrate (PHB), and in Japan: chitin from shrimp, zein from corn. Another research conducted is how to obtain degradable thermoplastic packaging that has a relatively longer lifetime at a lower price.[4]

Indonesia is a country with great potential to produce bioplastics with the potential of its natural resources. One of them is by developing biopolymers from cellulose. Some of the plants contain cellulose compositions which are effective for use as plastic biopolymers such as corn cobs, banana peel, sweet potato peel and several other plants that have been used as a bioplastic base material.[5] The cassava peel waste is used as a material for making bioplastics as a way of dealing with recycled waste, knowing that the majority of cassava peel from the producer of Getuk (Javanese traditional wet cake made from pounded cassava) has been left unused so far.

The use of bioplastics for food packaging will be safer from the chemical aspect because the materials used are natural-based resourced, while the physical and microbiological parameters need to be studied because they are related to the characteristics of bioplastics which is physical and microbiological quality of food.[2] Based on that, the authors are interested in examining the effect of physical and microbiological quality of food in the use of bioplastics from cassava peel as food packaging.

MATERIALS AND METHODS

The type of research used is an experiment research, using a research design of a posttest with control. The research design used was a randomized block design.[5]

The formula used to determine the number of repetitions of treatment in this study is based on the calculation of the Gomes formula as follows[6]:

Formula : $t(r-1) \geq 15$

Description : t = Number of Treatment

r = Number of Repetitions

$t(r-1) \geq 15$

$3(r-1) \geq 15$
$3r-3 \geq 15$
$3r \geq 18$
$r \geq 6$

The conceptual framework in this study includes independent variables (the ratio of chitosan to glycerol with a mixture of 3% weight: 3 ml, 5% weight: 5 ml, and 7% weight: 7 ml respectively) and the dependent variable (physical and microbiological quality of food), while there are several confounding variables that can affect the results of the research, thus the confounding factor must be controlled including contact time, temperature, humidity, the thickness of the bioplastics, and the methods of making getuk. The research variables can be seen in the chart below:

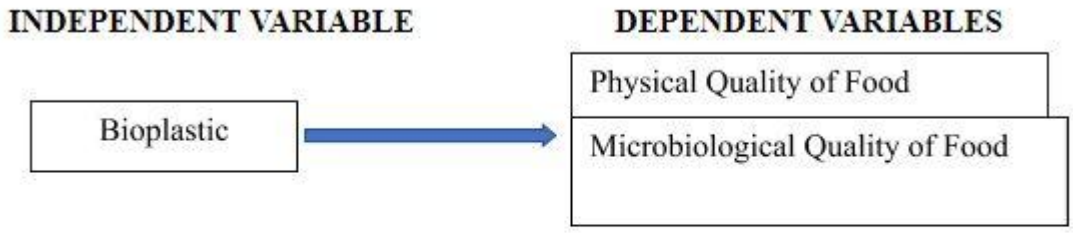


Image 1. Conceptual Framework on the Effect of Physical Quality and Microbiology Quality of a Food Packed Using Cassava Peels-Based Bioplastics

The population is the processed food in the form of getuk. The sample is a part of getuk taken from the place where getuk is made. Sample size = ((Treatment x Repitition) + Control) x Parameter = ((3 x 6) + 6) x 1 = 24 sample.
While the sample for organoleptic examination was 3 samples with 1 control.

Tools : Gas stove, blender, filter, beaker glass, stirring rod, scale, pipette, oven, incubator, petri dish, bioplastic printing equipment.

Ingredients: Vinegar, NaOH, chitosan, glycerol, PCA and getuk. organoleptic test observation instrument.

To make decisions from the data obtained in this study, data analysis was carried out using univariate and bivariate (ANOVA) analysis. 7 Univariate analysis is also called descriptive analysis, which is an analysis that describes in detail the characteristics of each of the variables studied. For numerical data, each variable can be described based on its middle size (mean, median, and mode), actual size (minimum value, maximum value, standard deviation, bivariate, and linear quartile range).

The numerical data in the descriptive analysis was based on the mean value and the value of the data distribution. The mean that is used depends on the distribution of the data. For data that is normally distributed, the middle value used is the mean, while for data that is not normally distributed, the middle value used is the median value. While the commonly used data distribution values are the minimum and maximum values, and the standard deviation values. It is also necessary to display the value of the Confidence interval (95% CI).

Physical parameters measured by the organoleptic test were analyzed descriptively based on the opinions of 3 panelists. The statistical test used to analyze the difference of more than two means is by using the one-way ANOVA test in this study. ANOVA test was used to determine the differences in the effect on the physical and microbiological quality of food by adding chitosan and glycerol to bioplastics made from cassava peel as food packaging.

Bivariate analysis was used to see the mean difference between the data for more than 2 groups. The principle of the ANOVA test is to analyze the variability of data from two sources of variation, namely data from groups (within variations) and data on variations between groups (variations between). If the comparison between the variation between and within is 1 (the variation between and within is the same), there is no average difference between groups. On the contrary, if the ratio of variation between and within > 1, thus there is a difference in the average between groups.[7,8]

Statistical test using a software on a computer will display the p-value (p-value). The decision rule uses a probabilistic approach, which is to compare the p-value with the α (alpha) value.[8]

RESULTS

The process of making getuk starts from peeling the cassava and molding it into pieces of getuk that fits and suitable to be sold in the market. After peeled, the cassava is washed clean and then boiled for about 1.5 hours until cooked, then the boiled cassava is grounded so that it becomes a getuk dough with white sugar added, then it is molded according to the size of getuk which is 7 cm x 5 cm with an average weight of getuk as 58, 8 grams per piece, the process that can cause a risk of contamination on the getuk was when the getuk is left open in one room before wrapping it in plastic. The results of the examination of getuk wrapped in plastic on the second day showed the results of 2163 CFU/gram, this means that the getuk on the second day still met the requirements for the total plate count of bacteria because the standard was 1 x 10^4 CFU/gram.

One of the goals of using cassava peels for making bioplastics is to reduce organic waste from cassava peels, we can produce up to 415 grams of bioplastic essence from 10 kg of cassava peels waste. After processing the cassava peel with the addition of chitosan and glycerol, it will become a gelatin that is ready to be molded into bioplastics, an average of 40 grams of gelatin can be molded into bioplastics with a size of 30 cm x 20 cm.

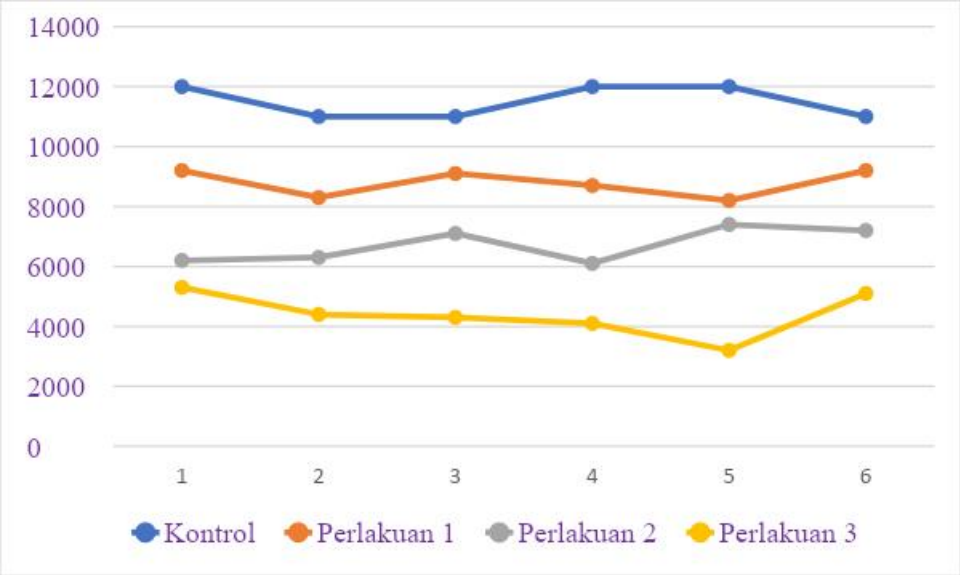
The time of research was from April 28, 2017 to May 31, 2017. The shelf life of getuk is two days, thus the sample is examined on the third day. The results of the Total Plate Count (TPC) on the getuk packed with bioplastics in the control group, in treatment 1, treatment 2, and treatment 3 can be seen in table 4.1 below:

Table 1. The Examination results of the Total Plate Count (TPC) on Getuk Packaged with Bioplastic

Repetition	Total Colony of Bacteria (3rd Day Examination)			
	Control	3% : 3 ml	5% : 5 ml	7% and 7 ml
1	$1{,}2 \times 10^4$	$9{,}2 \times 10^3$	$6{,}2 \times 10^3$	$5{,}3 \times 10^3$
2	$1{,}1 \times 10^4$	$8{,}3 \times 10^3$	$6{,}3 \times 10^3$	$4{,}4 \times 10^3$
3	$1{,}1 \times 10^4$	$9{,}1 \times 10^3$	$7{,}1 \times 10^3$	$4{,}3 \times 10^3$
4	$1{,}2 \times 10^4$	$8{,}7 \times 10^3$	$6{,}1 \times 10^3$	$4{,}1 \times 10^3$
5	$1{,}2 \times 10^4$	$8{,}2 \times 10^3$	$7{,}4 \times 10^3$	$3{,}2 \times 10^3$
6	$1{,}1 \times 10^4$	$9{,}2 \times 10^3$	$7{,}2 \times 10^3$	$5{,}1 \times 10^3$

Based on table 1, the total plate count in the control group obtained the lowest results of 1.1×10^4 colonies and the highest was 1.2×10^4 colonies, in treatment 1 the lowest results was 8.2×10^3 colonies and the highest was 9.2×10^3, in treatment 2 the lowest results was 6.2×10^3 colonies and the highest was 7.4×10^3, while in treatment 3 the lowest results was 3.2×10^3 colonies and the highest was 5.3×10^3.

The results showed that the total plate count of bacteria from a sample of getuk packed with bioplastic in the control group was higher when compared to the total plate count of bacteria in treatment group 1, the total plate count of bacteria in treatment group 2, and the total plate count of bacteria in treatment group 3, as seen in the graph below:



Graph 1. Total Plate Count (TPC) on Control and Treatment

The results of the physical examination consisting of the aroma and texture of getuk carried out by 3 panelists were as follows:

Table 2. Results of Organoleptic Test on Getuk packed with Bioplastics (3rd Day Observation)

Organoleptic Test	Dosage of Chitosan and Glycerol			
	Control	3% : 3 ml	5% : 5 ml	7% and 7 ml
Aroma	Smells slightly sour	The aroma of getuk does not release odor, the aroma of getuk remains the same.	The aroma of getuk does not release odor, the aroma of getuk remains the same.	The aroma of getuk does not release odor, the aroma of getuk remains the same.
Texture	The texture of the getuk turns a little sticky (slimy) and the edges that are not wrapped Hardens	Non-sticky texture	Non-sticky texture	Non-sticky texture

Based on the organoleptic test, the results of the quality in the control group of getuk, both aroma and texture, tended to not meet the requirements, because it smelled slightly sour and the texture was sticky/slimy, while the treatment group was still in a good condition because the aroma remains the same as how it was in the second day and the texture was not sticky.

Univariate Analysis

Univariate data analysis was used to determine the mean, minimum and maximum value, 95% CI and the standard deviation value of the Total Plate Count (TPC) obtained in control, treatment 1, treatment 2 and treatment 3.

Tabel 3. Average Distribution of Total Plate Count on Getuk Packaged with Plastic

Variable	Mean	SD	Min-Max	95% CI
Total Plate Count	7850	2729,23	3200 - 12000	6697,55 – 9002,45

Based on table 3, the results of the analysis showed that the total plate count was 7850 colonies (95% CI: 6697.55 - 9002.45), with a standard deviation of 2729.23 colonies. The lowest number of colonies was 3200 colonies and the highest number of colonies was 12000 colonies. Based on the results of interval estimation, it can be concluded that 95% is believed that the average number of colonies is between 6697.55 - 9002.45 colonies.

Bivariate Analysis

Bivariate analysis was used to determine the differences in the average number of total plate count in treatment 1, treatment 2, and treatment 3.

Table 4. Average Distribution of Total Plate Count on Getuk Packaged with Plastic

Variable	Mean	SD	95% CI	p-Value
Control	11500.00	547.723	1095,20-12074	0,00
Treatment 1	8783.33	453.505	8307,41-9259,26	
Treatment 2	6716.67	577.639	6110,47-7322,86	
Treatment 3	4400.00	753.658	3609,08-5190,92	

Based on table 4, an average of the total plate count in the control group is 11500 colonies, in treatment 1 the average total plate count was 8783.33 colonies, in treatment 2 the average total plate count was 6716.67 colonies, and in treatment 3 the total plate count was 4400 colonies. While ANOVA test results obtained p-value = 0.00, meaning that there is a significant difference in the microbiological quality of food using bioplastics from cassava peels as a food packaging.

To find out at which concentration the difference in total plate count of getuk, thus ANOVA test was followed by the post hoc test, namely the Bonferoni test. The results can be seen in the following table:

Table 5. Post Hoc Test Results The Effect of Using Bioplastics from Cassava Peel as Food Packaging on Food Microbiological Quality.

Treatment	p-Value
Control with Treatment 1	0,000
Control with Treatment 2	0,000
Control with Treatment 3	0,000
Treatment 1 with Treatment 2	0,000
Treatment 1 with Treatment 3	0,000
Treatment 2 with Treatment 3	0,000

Based on table 5, it has shown a significant result on the microbiological quality of food using bioplastics from cassava peels as food packaging, which include a result of control, treatment 1, treatment 2, and treatment 3 of p-value = 0.000, while in treatment 1 there is a significant difference with treatment 2 and treatment 3 with a value of p = 0.000, and treatment 2 with treatment 3 with a value of p = 0.000.

Table 6. Results of Homogeneous Subsets on the effect of microbiological quality in using bioplastics from cassava peel as food packaging.

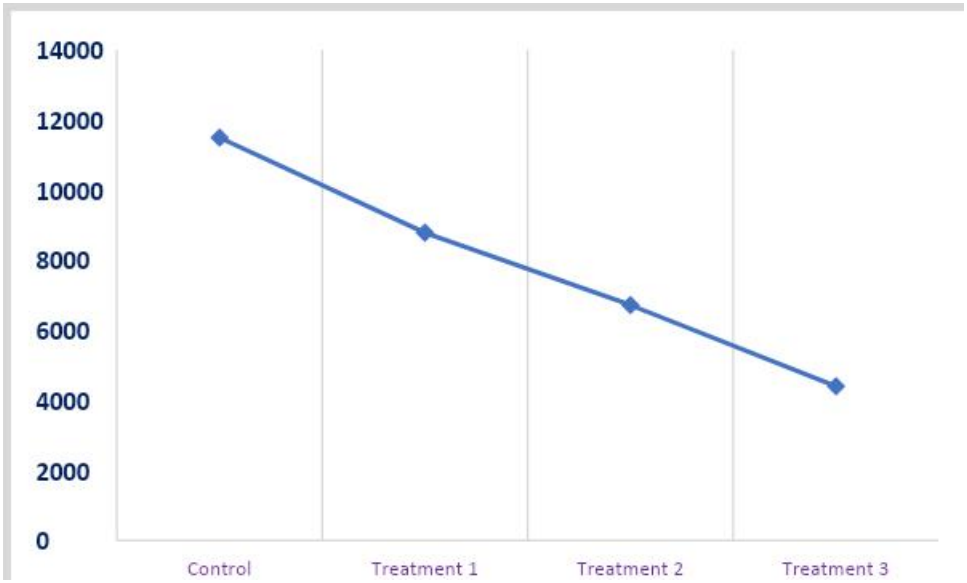
Addition of Chitosan and Glycerol	Subset for alpha = 0,05			
	1	2	3	4
Control				11500
Treatment 1			8783,33	
Treatment 2		6716,67		
Treatment 3	4400			

Based on table 6, the results showed that in treatment 1, the microbiological quality of food by using bioplastics from cassava peels as food packaging can maintain the microbiological quality of getuk within an average of TPC 11500 CFU/gram. in treatment 1 it can maintain the microbiological quality of getuk with an average of TPC 8783.33 CFU/gram, in treatment 2 it can maintain the microbiological quality of getuk within an average of TPC 6716.67 CFU/gram, and in treatment 3 it can maintain the microbiological quality of getuk within an average of TPC 4400 CFU/gram. Treatment 3 showed the lowest amount of TPC in the microbiological quality of getuk in this study.

DISCUSSION

The quality of the getuk which was packed with plastic/ the control group showed that the physical quality was not good on the third day because it smelled sour and the texture of the getuk became a little sticky or slimy. The condition of the getuk that smells sour and its slimy texture indicates that the getuk has been damaged. This damage can be caused because the plastic used were unable to protect the getuk thus it comes into contact with the air. The damage is also caused by the activity of microorganisms. This is indicated by the results of the average total plate count (TPC) examination of getuk, which is 11500 CFU/gram. The cause of the high TPC in plastic/ control packed getuk was because the plastic packaging does not absorb water so that the water level was high enough that it causes smell and texture of getuk to experience damage/change.

The average value of the Total Plate Count (TPC) of getuk packed with bioplastic can be seen in the graph below:



Graph 2. The Average of TPC on Control and Various Treatment

The average total plate count in treatment 1 was 8783 CFU/gram, in treatment 2 was 6716 CFU/gram and in treatment 3 was 4400 CFU/gram. Based on these results, the more glycerol and chitosan added, the lower the total plate number, with the required contamination limit for getuk which is 10,000 colonies /gram, this means that the control group does not meet the requirements while in the treatment group 1, treatment group 2 and treatment group 3 has fulfilled the requirements.

The physical quality of getuk which is packed with bioplastics made with the addition of glycerol and chitosan does not resulted in bad condition. Based on observations until day 3, the aroma and texture of getuk packed by bioplastics did not change in aroma and texture. This is because glycerol is hydrophilic and chitosan is microbial thus it can suppress the growth of

microorganisms. Hydrophilic glycerol causes food not to accumulate moisture from respiration which can affect the growth of microorganisms.

Based on the results of the study, the control group obtained the lowest results of 1.1 x 10^4 CFU/gram and the highest was 1.2 x 10^4 CFU/gram, the high TPC was caused by the nature of the plastic packaging which could not absorb water/was not hydrophilic so that the growth of microorganisms could occur. The TPC did not meet the requirements because it had exceeded 1 x 10^4 CFU/gram.

Where as in treatment 1 (3% chitosan and 3 ml glycerol) the lowest result was 8.2 x 10^3 colonies and the highest was 9.2 x 10^3, these results have met the quality standards of getuk TPC. The nature of bioplastics is quite elastic and flexible and it can absorbs water vapor from the outside air which contains bacteria so that the growth of microorganisms can be suppressed. The results in treatment 2 (5% chitosan and 5 ml glycerol) obtained the lowest results of 6.2 x 10^3 colonies and the highest was 7.4 x 10^3, while in treatment 3 (7% chitosan and 7 ml glycerol) the lowest results was 3.2 x 10^3 colonies. and the highest were 5.3 x 10^3.

The results in treatment 2 and treatment 3 have met the TPC quality standard. Treatment 3 shows the lowest total plate count, this proves that the more glycerol and chitosan are added, the lower the total plate count, in treatment 3 the bioplastic properties that are formed are more hydrophilic so that the higher the ability to absorb water vapor from outside air. This causes the growth of microorganisms to be inhibited due to the low water content of the getuk.

ANOVA test results obtained p-value = 0.00, meaning that there was a significant difference in the use of bioplastics from cassava peels as food packaging to the microbiological quality of food. This showed that besides being hydrophilic, bioplastics can also be used as antimicrobials, this result is in line with previous research, which is bioplastics made from cassava peels added with glycerol alone can reduce the total plate count (TPC) in dodol food packed with bioplastics with a result of 145 CFU/gram.

The quality of the bioplastics produced is slightly cloudy, this is because the starch of the cassava peel is brown and there is a change in color when the raw material for the essence of the cassava peel is left in contact with outside air for a long time.[9] The addition of glycerol and chitosan is expected not to be harmful for human's health. The purpose of adding these materials is to improve the elasticity and flexibility of the bioplastics produced. Based on the research results, the dose of 7 ml gave the best effect on TPC in getuk.

Glycerol usage regulations state that glycerol is a safe additive for consumption. Glycerin (E422), used as an additive in moisturizers, candy, dried fruit and low-calorie foods. Glycerol Acceptable Daily Intake (ADI) is not specified, glycerol recommendation according to ADI is not specified. However, based on FDA-21 CFR 182.1320, glycerol is classified as GRAS (Generally Recognized As Safe) if its use is in accordance with GMP (Good Manufacturing Practice) standards.[10] Ingesting 100 ml of Glycerol can cause headaches, nausea and vomiting. Other symptoms include gastrointestinal irritation, insomnia, dizziness, diarrhea, and fever. Ingesting 30 ml of glycerin for 50 consecutive days causes an increase in thirst and feelings of warmth.

The addition of NaOH is intended to form alkaline properties of dough thus it could easily remove bioplastics from the mold equipment. It would be very dangerous in case of skin contact (corrosive, irritant, permeator), eye contact (irritant, corrosive), ingested, or inhaled. However, it was only used in a little amount, which is only a few drops until the acidity level of the cassava peel essence mixture meets the requirements.

One of the advantages of bioplastics is that they are easily biodegradable, while plastics made from petroleum which is used worldwide up to 100 tons/year can have a negative impact on health, namely plastics containing bisphenol A at low doses can have an impact on increasing prostate levels, decreasing testosterone levels, increase the risk of breast cancer, prostate cells become sensitive to hormones and cancer, it can also make a person hyperactive.[10] Therefore, the use of a very large amount of plastic can have a significant impact on human health and the environment because the plastic properties are difficult to degrade, it is estimated that plastic will take 100 to 500 years to completely decompose.[11] The biodegradable properties of bioplastics is very suitable for alternative use of wrappers, especially for food packaging. The time required for the complete degradation of the bioplastic is only about 15 days.[4]

CONCLUSION

The physical quality results of a food packed in bioplastics from cassava peels a on the third day has showed that in the control group the aroma of getuk became slightly sour and the texture was a bit slimy/sticky with hardened edges, while in treatment 1, treatment 2 and treatment 3 the resulting aroma of getuk does not release odor and its texture remains the same as how it was in the second day (non-sticky texture).

The utilization of bioplastics from cassava peels as food packaging to the microbiological quality of food resulted in the average of total plate count at 7.85 x 10^3 CFU/gram, with a standard deviation of 2.729 x 103 CFU/gram. The lowest number of colonies was 3.2 x 10^3 CFU/gram and the highest number of colonies was 12 x 10^3 CFU/gram. Based on the results of interval estimation, it can be concluded that 95% is believed that the average number of colonies is between 6.697 x 10^3 CFU/gram - 9 x 10^3 CFU/gram.

There is a significant difference in the use of bioplastics from cassava peels as food packaging on the microbiological quality of food, with a value of $p = 0.000$.

SUGGESTION

Cassava peel waste can be used as a basic material for making bioplastic, Bioplastics with the addition of chitosan and glycerol can be used as an alternative in food packaging because it can reduce the total plate count of a bacteria and can maintain the physical quality of getuk. Further researches in the quality of bioplastics are needed so that more pieces of information are known regarding biodegradation time and other types of microbiology.

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