RESEARCH ARTICLE

BEKICOT (ACHATINAFULICA) MICROBIOLOGY TEST BASED ON THREE WAYS TREATMENT AND STORAGE DURATION

Mery Pattipeilohy[1], Stevin Melay[1] and Stenly Lessu[2]

1. Biology Education Study Program, Faculty of Teacher Training and Education, Pattimura University of Indonesia.
2. Alumni Biology Education Study Program, Faculty of Teacher Training and Education, Pattimura University of Indonesia.

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Manuscript Info	*Abstract*
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Bekicot (Achatinafulica) is a gastropod class animal consumed by TNS community. It is processed in several ways including fried, boiled, and roasted. This study aims at determining the amount of bacteria found in snails that are processed in accordance with the habits of the community and stored for 3 hours, 6 hours, and 9 hours. This type of research is a descriptive study to see the quality of bicrobiology. This research was conducted in TNS sub-district for sampling and a microbiology test was carried out in the Basic Laboratory of Biology Education at Pattimura University. The results showed that among the three treatments, the lowest number of colonies was 3 hours treatment. The lowest amount of bacteria was found in bekicot meat by roasting with an average of 11.3 Cfu / g. In the storage period of 6 and 9 hours the meat is not safe to be consumed because it contains more than 5x105 Cfu / g bacteria, the highest number of bacteria or cannot be counted (CNbC) is found in meat by boiling.

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Introduction:-
As the basic necessity for human being, it is advised to consume fresh food either from plants or animals (1, 12, 19).

Bekicot (Achantinafulica), a gastropod class snail is one of the food sources contain high nutritious protein.(3, 6). This species lives in agricultural areas, coastal areas, wetlands, natural forests, shrubs, and urban areas. Besides, it can also live in forests, and plantations or cultivation sites (4, 16). This land snail is abundant in times of high rainfall in tropical regions. A. fulica is the main plant pest species

which originated from East Africa but it hasspread all over the world since before the 1800s (2).

Bekicot has high protein. The protein content of this snail is 59.28%, fat3.62%, crude fiber 2.47%, calcium 6.4% and phosphorus 0.85% (9). However, this snail is poisonous and can causedeath if nottreatedproperly.

This snail has been consumed by people in Central Maluku District, especially in TNS Sub-district. The meat of the snail can beprocessed in various ways such as frying, roasting and boiling without adding preservatives. In other areas, it is made into crackers or satay. By frying, roasting and boiling, microorganisms can contaminate the meat, it

Corresponding Author:- Mery Pattipeilohy
Address:- Biology Education Study Program, Faculty of Teacher Training and Education, Pattimura University of Indonesia.

is becausethe processed meat is not consumed all at once but there is leftover to be consumed later. The leftover contains water that might cause microorganisms to activate enzymes that play a role in the process of biodegradation of proteins that can cause decay(14). Therefore, storage of processed meat of the snail should not exceed 4 hours after being cooked until consumed (13). It is necessary to conduct research to determine the presence of microbes in three types of processing and different storage times.

Besides being a source of nutrition for humans, food is also the source of food for microorganisms.The growth of microorganisms in food can be beneficial or detrimental. For example, it can benefit to the improvement of nutrition, digestibility or storage. However, it can also cause undesirable physical or chemical changes, so the food is not suitable for consumption (11, 12).

Materials and Method:-
This research applies descriptive studyapproach using a completely randomized design(CRD) that is to see the number of bacteriain bekicot meat that are processed by boiling,smoking, and fried and carried out withdifferent storage times,namely 3hours, 6hours and 9 hours.

Research Procedure:
Sterilization of Tools and Materials:
Sterilization was done using hot steam sourced from the autoclave at a temperature of 120o C at a pressure of 15 psi. This is to avoid contaminants by bacteria that are not from the sample used.

Creating the Medium:
Preparation of peptone (liquid) medium for dilution by weighing 15 grams of peptone and put in 1000 ml of distilled water into an erlemeyer flask and then heated on a hotplate until it becomes homogeneous. The medium is ready to be sterilized in an autoclave for 15 minutes. Medium Nutrient (solid) for bacterial growth by weighing 14 grams of NA and 500 ml of distilled water, into an erlemeyer flask, then heat it on a hotplate until the solution becomes homogeneous. The medium is ready to be sterilized in an autoclave for 15 minutes.

Sampling Stages:
Snail sampling was taken from TNS District. Snails selected have the same size as the sample pliers taken directly put into a bucket and covered with plastic. Snail samples then are ready to be taken to the Biology Lab for treatment

Sample Preparation:
Snail meat is separated from the shell. After being separated, washed and soaked with 15% salt water for 60 minutes so that the mucus contained in the snail is easily removed.

Processing Process:
The processing of bekicotmeat is done in 3 different times. This is because a preliminary test of the processing time has been done for the three treatments. For boiling it takes 25 minutes, while roasting andfrying 5 minutes.However,the focus of this study is to see length of storage for 3 hours, 6 hoursand 9 hours.

Dilution Stage:
Snail meat samples treated using three treatments were taken as much as 10 gramsfrom the three treatments to conduct the dilution stage.The dilution step iscarried out at a dilution rate of 10-1, 10-2,10-3 and the sampleused for inoculation isat a 10-3 dilutionlevel which will beimplanted in the Natrium Agar (NA) mediumtaken as muchas 0.1 ml.

Planting:
The method used is the agar surface method (spread plate). The media is poured into a petri dish and allowed to freeze. After freezing perfectly, samples were taken usinga 0.1 ml pipette from a 10-3 dilution and spread into petri dishes which already contained NA media. After that each petri dish is given a name, level of dilution andtest date, the petri dish is then put into an incubator and incubated for 24 hours at 370C.

Observations:-
After incubationthe petri dishes areremovedand arranged accordingto the dilution level. Numbers of colony in each petri dish iscounted using colony counter.

Techniques of DataAnalysis:
The data obtained in this study are descriptive to see the number of microbes contained in a Petri dish.

Data Collection Procedure:
Data collection was carried out after an incubation period of 1 x 24 hours. Bacterial colonies are counted in each petri dish.Calculation of the number of bacterial was done using a colony calculation tool. The number of bacterial colonies was counted in each cup, then the number of bacterial colonies were counted per gram sample as specified. Number of colonies = number of colonies per cup

$$x\frac{1}{fak.dilution} x10$$

For microbial analysis a standard called "standard plate count (SPC)" is used.

Results And Discussion:-
Results:
Testing the presence of bacteria in bekicot inoculated by using the Surface pour Plate (7) method, while calculating bacterial colonies by using a Colony Counter, each cup viewed the total bacterial colonies per gram of bekicotsamples (1). Microbiological quality testing aims to determine the total number of bacterial colonies in each fumigation material. Observation data on the processing with length of storage towards the presence of bacteria in bekicot meat are presented in table 1, 2 and 3 below:

Table 1:- Bekicot processing method for 3hours storage.

No	Processing method	Dilution	ΣColony of bactery (Cfu/g)	Totalplate count (Cfu/g)	Average
1	Fried	10^{-3} 10^{-3} 10^{-3}	8 22 11	$8,0 \times 10^4$ $2,2 \times 10$ $1,1 \times 10^5$	13,6 Cfu/g
2	Roasted	10^{-3} 10^{-3} 10^{-3}	20 6 8	$2,0 \times 10^5$ $6,0 \times 10^4$ $8,0 \times 10^4$	11,3 Cfu/g
3	Boiled	10^{-3} 10^{-3} 10^{-3}	23 14 10	$2,3 \times 10^5$ $1,4 \times 10^5$ $1,0 \times 10^5$	15,6 Cfu/g

Table 2:- Bekicot processing method for 6 hours storage.

No	Processing method	Dilution	ΣColony of Bactery (Cfu/g)	Total plate count (Cfu/g)	Average
1	Fried	10^{-3} 10^{-3} 10^{-3}	53 74 25	$5,3 \times 10^5$ $7,4 \times 10^5$ $2,5 \times 10^5$	51 Cfu/g
2	Roasted	10^{-3} 10^{-3} 10^{-3}	44 54 79	$4,4 \times 10^5$ $5,7 \times 10^5$ $7,9 \times 10^5$	59 Cfu/g
3	Boiled	10^{-3} 10^{-3} 10^{-3}	56 64 TBUD	$5,6 \times 10^5$ $6,4 \times 10^5$ CNbC	420 Cfu/g

Remarks:CNbC = Can not be counted

Tabl 2 3:- Bekicot processing method for 9 hours storage.

No	Processing method	Dilution	ΣColony of	Total plate count	Average

			Bactery (Cfu/g)	(Cfu/g)	
1	Fried	10^{-3} 10^{-3} 10^{-3}	TBUD 24 48	TBUD $2,4 \times 10^5$ $4,8 \times 10^5$	124 Cfu/g
2	Roasted	10^{-3} 10^{-3} 10^{-3}	40 8 TBUD	40×10^5 8×10^5 TBUD	116 Cfu/g
3	Bioled	10^{-3} 10^{-3} 10^{-3}	TBUD TBUD TBUD	TBUD TBUD TBUD	CNbC

Remarks :CNbC = Can not be counted

The results of observation in table 3 shows that the number of colonies that grow exceeds the maximum limit of 5x105 Cfu / g and is not suitable for consumption and the highest number of CNbC bacteria was found in bekicot's meat by boiling method, followed by roasted method with an average of 116 Cfu / g, and fried method with an average of 124 Cfu / g.

Discussion:-

Bekicot meat processing was done with 3 different treatments and inoculated on NA medium used Spread plate method at 10^{-3} dilution. The 10^{-3} dilution process was aimedat reducing or minimizing the number of microbes suspended in the liquid (19). From the results of the study,it was obvious that physical form (organoleptically) in bekicot meat from the three processing methodsis still excellent, but based on microbiological test results not all snail meat can be consumed.

3 hours storage time is the best storage time used to store bekicot meat because it produces the least amount of total bacterial colonies compared to 6 hours and 9 hours of storage. So that the storage time of 3 hours for bekicot meat is highly recommended for consumption either by frying, roastingor boiling because the growth of bacteria in petri dishes has not exceeded the maximum limit set by the SNI which is 5x105 per cup (table 1), whereas in fried,roasted, and boiled bekicot meat for 6 and 9 hours of storage and incubated for 24 hours found that the bacteria grew beyond the maximum limit, making it unsafe to be consumed (table 2 and 3).

The presence of bacteria in bekicotmeat with 6-9 hours of storage is due to high water content in the meat. The damage to the meat is caused by microorganisms, enzyme activity, temperature, and water content which is affectedby the duration of storage, that will cause greater damage. This is because the bases broken down by bacteria get more and more over time (10, 12, 16)

Besidesfish, bekicot is an important source of nutrition for humans because they are rich in protein. The advantage of bekicotas food is because it is easy to get in the environment and accessible by the community and it is easy to digest as well. However the food products consumed by humans are stronglyinfluenced by microorganisms (15). In addition, microbes can contaminate food through water, dust, air, soil, processing equipment (during the production orpreparation process) as well as secretionsfrom the intestinesof humans oranimals(17).

Pathogenic bacteria can grow and multiply during storage, so that they are able toproduce toxins that can be harmful to humans (6), so that storage should notexceed 4hours(13).

One of the factors that influence the growth of microorganisms that destroy fresh food stored is temperatureand water (8, 9, 14). Materials that are dissolved in water, can be used bymicroorganisms to form cellmaterial andobtain energy (12).

Food damage by microorganisms is a form of damage that is often harmful and sometimes harmful to human health, because the toxins produced can be consumed by humans (7, 12).Enterobacteriaceae is often found in smoked fish that are high in water content, this willalso occur in bekicotbecause the water content in these three treatments is also quitehigh. Because processing methods combined with heating and contamination fromprocessors are unavoidable, bekicot processed products are also susceptible to the growthof Staphylococcus aureus, Aspergillus sppandPenicilliumspp(11).

In addition to diseases caused by bacteria, another danger is the occurrence ofpoisoning due to the growth of Clostridium botulinum, a spore-forming bacteria that isvery heat-resistant,which producesbotulismpoison(10).

Based on the results of this study, it is recommended that bekicot's meat that is fried, roasted and boiled shouldbe consumed immediately after processing because it has not been much contaminatedby bacteria. If it is to be stored, it is better if the storage time is no more than 4 hours, sothat it is still safe to be consumed or it is recommended to store the meat at lowtemperatures such as refrigeratorsbecause low temperatures can slow food spoilage orenzymatic processes caused by microorganisms.

Conclusion:-
Based on the results of the study it can be concluded that the method of processingby frying,roasting and boiling affects the presence of bacteria, therefore a goodstorage time is 3 hours because the number of bacterial colonies does not exceed themaximum limit of microbial contamination issued by the Director General of Drug andFood Control is 5,104 cpu / gram

Suggestions:-
This research needs to be continued to see species of contaminant bacteria found in snails.For TNS people, it is better toconsume bekicot meat that is not more than 3 hours storage time.

References:-
1. Buckle, K. A., Edwards, R. A., Fleet, G. H., and Wotton, M. 1987. IlmuPangan. Penerjemah Hari PurnomodanAdiono. Universitas Indonesia Press. Jakarta.
2. Bhattacharyya.B. Mrinmoy Das. HimangshuMishra. D.J. Nath and SudhansuBhagawati. Bioecology and management of giant African snail,Achatinafulica (Bowdich). International Journal Of Plant Protectionvolume 7 | Issue 2 | October, 2014 | 476-481
3. Campbell, N.A., Jane B.R., danLawerance G.R., 2000. Biologi.Erlangga.Jakarta. Hal. 224-225.
4. Dewi. Prastiana. S. PerbedaanEfekPemberianLendirBekicot (Achantinafulica) dan Gel BioplacentonTerhadapPenyembuhan Luka BersihPadaTikusPutih [Skripsi], FakultasKedokteran. 2010. UniversitasSebelasMaret.
5. Dwidjoseputro. 2005. Dasar-DasarMikrobiologi. Djambatan. Jakarta.
6. Eze, E. I., Echezona, B. C.1 and Uzodinma, E. C. 2011. Isolation and identification of pathogenic bacteria associated with frozen mackerel fish (Scomberscombrus) in a humid tropical environment. African Journal of Agricultural Research Vol. 6(8).
7. Fardiaz, S. 1993. AnalisisMikrobiologiPangan. PT. Raja GrafindoPersada, Jakarta.
8. Fardiaz, S. 1989. KeamananPangan. PT RajaGrafindoPersada. Jakarta.
9. Kompiang,L.P. 1979. PendayagunaanBekicot. Kongres Nasional Biologi IV. Bandung.
10. Margesin, R. 2008. Effect of temperature on growth parameters of psychrophilic bacteria and yeasts. Springer. DOI 10.1007/s00792-008-0213-3
11. M-T. Gekenidis, D. Gossin, M. Schmelcher, U. Sch€oner, M.N.P. Remus-Emsermann and D. Drissner . 2017. Dynamics of culturable mesophilic bacterial communities of three fresh herbs and their production environment. Journal of Applied Microbiology ISSN 1364-5072
12. Moss, 2000. Food Microbiology. University of Surrey, Guildford, UK.
13. Puspitaningtyas.R.UpayaPenjamahMakananDalamMenjagaKualitasDitinjau Dari Aspek Food Safety PadaWarungMakan Di SekitarUniversitasNegeriSemaran.JurusanIlmuKesehatanMasyarakat, FakultasIlmuKeolahragaan, Universitas Negeri Semarang. 2015 https://lib.unnes.ac.id/
14. Pattipeilohy M. PengaruhJenisBahan, WaktuPengasapan Dan Lama PenyimpananTerhadapDayaTahan Serta KualitasMikrobiologiIkanCakalang(Katsuwonus pelamis) Sebagai Sarana Penunjang Materi Pengawetan Makanan Dalam Matakuliah Mikrobiologi Pangan. Tesis. 2006. Tidak di publikasikan. Universitas Negeri Malang.
15. Pattipeilohy M. Moh. Amin. Umie Lestari. And EndangSuarsini. Isolation and identification of pathogenic bacteria In fresh tuna (katsuwonuspelamis) collected from Fishermen in ambon, Indonesia. Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 19, No. (4) : 2017 : 878-883
16. Raut, S.K. and Barker, G.M. AchatinafulicaBowdichand other Achatinidae as pests in tropical agriculture. In: G.M. Barker(ed.), Molluscs as crop pests. CABI Publishing, Hamilton, NewZealand, pp.55-114.2002
17. Waluyo. L. 2007. MikrobiologiUmum. UMM Press. Malang

18. Walker.S.J. 2000. The Principles and Practice of Shelf-life Prediction for Microorganism. Book of Shelf-life Evaluation of Foods. Aspen Publisher. Gaitherburg

19. Wasteson, Y, and Hornes, E. 2009. Pathogenic Escherichia Coli Found in Food. International Journal Of Food Microbiology, vol 12, Hal 103-114

20. Jeanne-Marie Membre. BenoLeporq. Miche`leVialette. Eric Mettlerc. Louise Perrier. Dominique Thuault. Marcel Zwieterin. Temperature effect on bacterial growth rate: quantitative microbiology approach including cardinal values and variability estimates to perform growth simulations on/in food. International Journal of Food Microbiology 100 (2005) 179– 186.

21. J. M. J. Mcclements. M. F. Patterson,and M. Linton. The Effect of Growth Stage and Growth Temperature on High Hydrostatic Pressure Inactivation of Some Psychrotrophic Bacteria in Milk. Journal of Food Protection, Vol. 64, No. 4, 2001, Pages 514–522.