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3rd IBOC International Biology Conference 2016

Biodiversity and biotechnology for human welfare

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Preface

Biodiversity of Indonesia has been acknowledged as one of the richest among other countries in the world. Until recently, biodiversity is still a hot topic and issue to be disseminated not only in scientific meeting, but also as a core of major subject that should be introduced in the early education in Indonesia. In addition to biodiversity, biotechnology is also a discipline of life science that has been implemented for future used of human welfare. For this reason, we still use a theme "Biodiversity and biotechnology for human welfare" for our third biannual series conference.
This biannual meeting is devoted for creating networks among our department and several institutions that have a similar biological science in implementing that theme. It has been known world environment's encounters problems because of natural and anthropogenic impacts that effects on human being. Some examples like global warming, reducing biodiversity and extinction of vulnerable organisms, vanishing environmental water, and land - air quality, are examples that has a serious impact human welfare. Through this meeting we would like to achieve many personal contacts, ideas, biological-environmental problem solve sharing and fruitful discussions in order to save the earth together. Therefore, we are really grateful and thankful that the participants who are interested to joint with are from the Philippine, Thailand, Singapore, Korea, Italia, and Indonesia.

The committee of 3rd International Biology Conference (IBOC), Biology Department Institut Teknologi Sepuluh November has received 95 articles and furthermore 43 full papers has been published in the American Institute of Physics (AIP) Scopus indexed conference proceeding of 3rd IBOC volume 1854, after reviewing process (http://aip.scitation.org/toc/apc/1854/1?size=20&expanded=1854). These 43 papers cover subject fields of Biomass and Bioenergy; Environmental Science and Ecology; Animal Science; Agricultural and Natural products. The rest of reviewed articles, which are 19 articles are published in this volume proceeding of 3rd IBOC

We would like also to thank to Surabaya-Indonesia to the Mathematics and Natural Sciences Faculty, for supporting the conference. The big remarkable applause is also going to our students who are giving their excellent hands for keeping the conference running on schedule. Last but not least, we have a big hope that a real excellent networking in the future may arise from this event.

Thank you and best regards.

Surabaya, July 15th 2017

Head of Biology Department
Dr. Dewi Hidayati

Content

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LC MS Determination of Java Ginseng (*Talinum paiculatum)* Ginsenoside

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ABSTRACT

This plant roots was used traditionally by boiling or brewed. However, the types of ginsenoside Java ginseng are unclear.LCMS was conducted to determine of ginsenoside type in both of hot water extract and ethanol extract. Both of hot water and ethanol extractwas rich of ginsenoside K, Rg2, Rg3, Rf, Rd, Re, Ro, Rb2, Rb3, Rc, and Rb1. Ginsenoside Rb1, Rb2, Rb3, Rc,and Rg1 are bioactive compound most commonly found both of hot water and ethanol extract. **. This shown that root steaming also contains ginsenoside even with low levels.**

Keywords: *Talinum paniculatum*, ginsenoside, antioxidant.

INTRODUCTION

Talinum paniculatum (Jacq) Gaertn (Portulacaceae) was known as Som Java. Indonesian peoples used *T. paniculatum* not only as anti-inflammatory and antioxidant but also as source of aphrosdisiac. The other hand, Java ginseng having anti-fertility for female and estrogenic activity (Thanamool *et al.*, 2013), enhance viability of sperm (Rahmi *et al.,* 2011), androgenic activity (Winarni, 2009), and anti-inflammatory (Sumastuti, 1999). Based on this potential, some Indonesians tribes refer to *T. paniculatum* as Java ginseng. Previous research has revealed that some tribes on the island of Java use root as one of a mixture of herbal medicine. *T. paniculatum* roots was washed, and then brewed with hot water or by boiling the roots. That water stew was used as a medicine (Wibowo *et al..* 2015).

Such as Korean and China, ginseng is one of the traditional herbal medicines that have many efficacies. Ginseng means healing (Gillis, 1997). Some ginseng is commonly used in Asian traditional herb medicine to treat a variety of diseases. Pharmacological effects of ginseng are related to with the effects which demonstrated in the cardiovascular system, central nervous system, and immune systems, endocrine, antineoplastic as well as on antioxidant activities (Attele, 1999; Leung and Wong, 2010; Lee and Kim, 2014). A wide scientific has reported that major active components in ginseng are the ginsenosides. There was a variation in the ginsenoside of different species of some ginseng (Huang, 1999).

Some ginsenoside have promising benefits in the health. Ginsenoside Rg1 has reported as a potential antioxidant for preventing of endothelial cell dis-function associated with athesclerosis via anti-oxidative and anti-apoptosis mechanism (Huang *et al.,* 2013). The other hands, Rg1 also reported has main function to induce regenerative repair of puerperal nerve injury and recovery nerve function (Wang *et al.,* 2014). The beliefs of some tribes that *T. paniculatum* is also their own ginseng and as an herb to cure some diseases need to be scientifically verified. However, the kinds of ginsenoside from *T. paniculatum* roots still unclear. The information on the types of ginsenoside is very important to provide right information in consumption safety of traditional medicinal. Therefore, the present study aims to investigate of ginsenoside type of *T. paniculatum* roots.

MATERIALS AND METHODS

Sample preparation for LCMS analysis

A mount of five samples 2 – 3 year old Java ginseng roots were collected from Plosoklaten, Kediri, East Java, Indonesia. The samples were authenticated in *UPT Balai Konservasi Kebun Raya Purwodadi LIPI* (Indonesia Institute of Science). The sample were cleaned, dried, and grinded up to be a fine powder. One g of sample was weighed accurately and extracted in 10 mL of aquadest in 90^{O}C in water bath for 3 hours. Then, samples keep at room temperatures for 24 hours under dark conditions. Then the extract solution was filtered by using a 0,22 mm filter membrane. Amount of 2µL aliquot was used for LCMS analysis. As comparison, the sample was also extracted with ethanol for overnight at room temperature.

LCMS analysis

T. paniculatum of ginsenoside profiling was performed by using Shimadzu LCMS - 8040 LC/MS (Japan) apparatus. Samples were separated on Shimadzu, Shim Pack FC-ODS (2 mm D x 150 mm, 3 µm) column at 35^{O}C temperature in flow gradient: 0/100 at 0 min, 15/85 at 5 min, 21/79 at 20 min, 90/100 at 24 min. The kinds of ginsenoside was determined and calculated quantitatively by using LCMS quantification mode method. Ammonium acetate 8 mM was used as main solvent under 90 min running time. All samples were analyzed by three replications and analyzed by descriptive.

RESULTS AND DISCUSSIONS

Type and levels of ginsenoside both of hot water and ethanol extract have been analyzed with LCMS. The ginsenoside profile is presented in Table 1. There were 13 types of ginsenoside was found in both of hot water and ethanol extracts. Based on the retention time, they were ginsenoside K, Rh2, Tg2, Rg3, Rf, Rg1, Rd, Re, Ro, Rb2, Rb3, Rc, and Rb1 in various concentration.There were 5 types of ginsenoside that have high levels of ginsenoside, i.e Rb3, Rb2, Rb1, Rc, and Rg1 respectively. The highest ginsenoside level was obtained in ginsenoside Rb3 reached at 21,193 and 3,743 mg/g dry weight respectively in both of ethanol and hot water extract. Ethanol extracts profiling amount ginsenoside higher than hot water extract. This indicates that root steaming also contains ginsenoside even with low levels.

Based on that description, both of ethanol and hot water extracts have the same ginsenoside type, but different ginsenoside levels. The data is also supported with the LCMS chromotogram profile Fig. 1. Based on Fig. 1, there are 13 types of ginsenoside both in both hot water extract and ethanol. Ginsenoside on hot water extract chromatograms (Fig. 1A) has different peak numbers with ethanol extract chromatograms (Fig. 1B) as well as different peak height. However, all the type of ginsenoside was detected at the same retention time (RT). This shown that there were 13 types of ginsenoside in both of hot water and ethanol extracts, but the levels of ginsenoside in each extract are different.

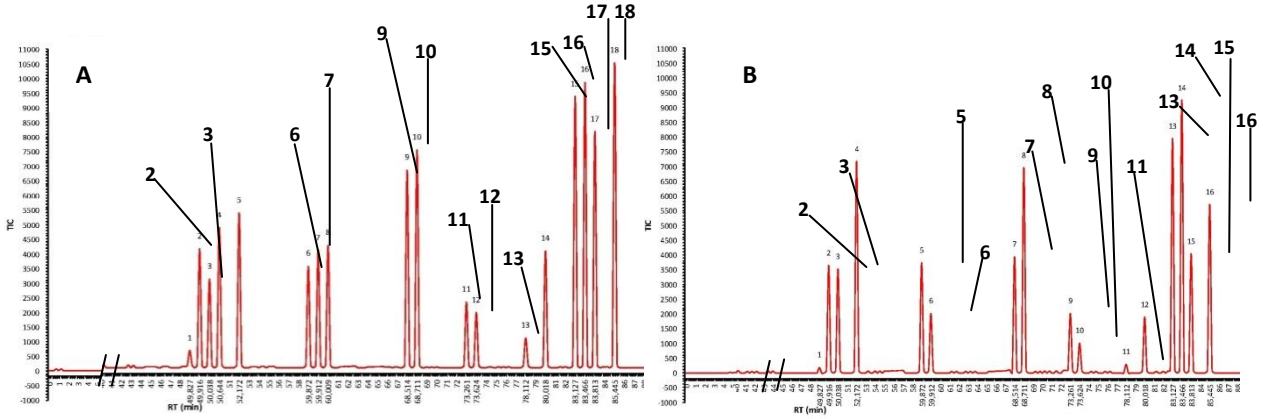

Figure 1. Comparison of ginsenoside compound chromatograms with GCMS on ethanol extract (A) and hot water (B).

Table 1. Profile of Ginsenoside*T. paniculatum* roots.

Num.	RT (min)	Type	Result (mg/g dried sampel)			
			Ethanol	Peak Num.	Hot Water (90^{O}C)	Peak Num.
1	49,916	Ginsenoside K	7,384 ± 1,384	2	1,520 ± 0,826	2
2	50,038	Ginsenoside Rh2	5,222 ± 0,505	3	1,816 ± 0,949	3
3	59,872	Ginsenoside Rg2	5,340 ± 0,674	6	1,308 ± 0,450	5
4	59,912	Ginsenoside Rg3	4,802 ± 0,940	7	0,656 ± 0,521	6
5	68,514	Ginsenoside Rf	9,156 ± 0,276	9	0,820 ± 0,144	7
6	68,711	Ginsenoside Rg1	11,574 ± 1,276	10	1,717 ± 0,260	8
7	73,261	Ginsenoside Rd	4,336 ± 0,813	11	0,603 ± 0,103	9
8	73,624	Ginsenoside Re	2,610 ± 0,117	12	0,263 ± 0,036	10
9	78,112	Ginsenoside Ro	2,032 ± 0,369	13	0,071 ± 0,023	11
10	83,127	Ginsenoside Rb2	17,746 ± 0,986	15	2,548 ± 0,218	13
11	83,466	Ginsenoside Rb3	21,193 ± 1,078	16	3,743 ± 0,441	14
12	83,813	Ginsenoside Rc	14,793 ± 0,697	17	1,519 ± 0,430	15
13	85,445	Ginsenoside Rb1	15,866 ± 0,931	18	1,364 ± 0,382	16

Ginsenosides can be divided to be three groups based on of their structure: the protopanaxadiol type, including ginsenosides Rd, Rc, Rb1, Rb2, Rb3, Rg3, Rh2, and others; the protopanaxatriol type, including ginsenosides Rg1, Rg2, Re, Rf and the others oleanane type (Hwang *et al.,* 2014 and Kim *et al.,*2008). But, The ginsenosides of Rg1 and Rb2 which were major components of the ginseng plants (Kim *et al.,* 2009). Each ginsenoside has different pharmacological effects. Previous studies have reported that ginsenoside Rg3 can prevent degeneration of dorsal neural neurons as well as an antioxidant effect on the corpus of cavernosum rats (Tao *et al.,* 2000)**.**Ginsenoside Rh2 can inhibited of growth and stimulated melanogenesis processes (Ota *et al.,* 1987) also arrested the progression of cell cycle at the G1 stage in melanoma cells (Ota *et al.,* 1997).The effects of ginseng on the cardiovascular system in particular can reduce the release of catecholamines caused by ACh from the adrenal chromaffin bovine cells (Tachikawa*et al.,* 1995). The multi-targeting ginsenoside may explain why Talinum has a wide range of beneficial effects so cause some tribes in Indonesia uses Talinum as one of traditional herb medicine.

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The Comparison of Males and Females Number of *Drosophila melanogaster* that Exposed by Mobile Phone in Multiple Generations

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ABSTRACT

Mobile phone is one of the wireless devices that emit electromagnetic radiation (EMF/EMR). *D. melanogaster* is one of the model organism that often used to reveal the effects of EMF on living beings.Myprevious study reportedthere were fluctuation of adult filial number and eclosion time of *D. melanogaster* that exposed by mobile phone in multiple generation.The purpose of this study was to investigate whether there were any difference between the number of males to females of *D. melanogaster* that exposed by mobile phone from first until fifth generation.Normal, *ebony body*, and *white eyes* strains of *D. melanogaster* from Genetic Laboratory FMIPA UM were used in this study. GSM mobile phone with provider using frequencies at 900/1800 MHz (3G) and HSDPA 2100 (4G) was used as a source of EMF exposure. In the results, the mean of Normal males number from first until fifth generation consecutively were 70.5; 52.5; 53.25; 124.5; and 54.0, while females were 64.0; 51.25; 54.25; 122.75; and 58.0.The mean of *ebony* males number from first until fifth generation consecutively were 41.0; 66.0; 65.25; 66.75; and 48.5, while females were 38.5; 61.0; 65.25; 73.75; and 49.25. The mean of *white* males number from first until fifth generation consecutively were 65.25; 54.5; 45.75; 62.25; and 38.75, while females were 61.5; 53.5; 49; 64.5; and 41.5. The results were evaluated for statistical differences by independent t-test if normality and homogeneity assumption were met and by Mann-Whitney U test if normality and/or homogeneity assumption were not met. Theconclusions, there were no significant difference (P>0.05) between the number of males to females at each generations of each strains.

Keywords: *Drosophila melanogaster,* EMF, mobile phone,sex ratio

INTRODUCTION

Mobile phone become more and more a necessary tool for human daily life (Panagopoulos and Margaritis, 2008). These tools help people to communicate easily with everyone at any place and any moment (Panagopoulos and Margaritis, 2008). Due to there are many benefits perceived by utilizing these tools, the number of mobile phone devices and duration of usage are increasing at a rapid rate (Huber *et al.,* 2002). However, although the mobile phone provides many benefits, there are serious threats arise from the exposure of these tools (Panagopoulos and Margaritis, 2008). All mobile phone emit a type of radiation called an electromagnetic field (EMF), composed of waves of electric and magnetic energy moving together through space (EHHI, 2012). Consequently, a rising number of persons are exposed to EMF in the radio-frequency range (Huber *et al.,* 2002).

Various studies have been conducted to determine the biological effects caused by EMFon various model organisms (Panagopoulos and Karabarbounis, 2004; Ruediger, 2009; Cucurachi *et al.,* 2013). Some researchs focused its study onthe relation of gender and respone of organism that exposed by EMF (Mirabolghasemi and Azarnia, 2002; Moorhouse and Macdonald, 2005; Belyaev, 2005). Those studies resulted various conclusions.

Relating to the duration of observation, various studies that have been mentioned just investigated the effect of EMF in just one generation.The study that evaluating the effects of EMF exposure at multiple generations is needed. Through such study, the long term effect of EMF exposure on population can be

assessed.In previous study, I demonstrated that the adult filial number and the eclosion time of *D. melanogaster* that exposed by mobile phone for five generations fluctuated from one generation to the next (Fauzi *et al.,* 2016). Yet, the effect of gender has not been evaluated in that study. Therefore, in this study, I addressed a new question, "Are there differences between the number of males to females of *D. melanogaster* that exposed by mobile phone from first until fifth generation?". Through this study, it is expected to obtain aninformation related to whether there are any difference radiosensitivity between male and female flies were exposed by mobile phone or not.

METHODS

The Organsim and Environmental Conditions

The Normal/*wildtype* (N), *ebony body*(*e*), and *white eyes*(*w*) strain of *D. melanogaster* from Genetics Laboratory FMIPA UM were used in this study (Figure 1). Flies were cultured in glass bottle with standard medium, as descibed in Fauzi *et al* [10] Fauzi *et al.,* (2016). The flies cultures were kept in a research room at 25.5 ± 1^{o} C.

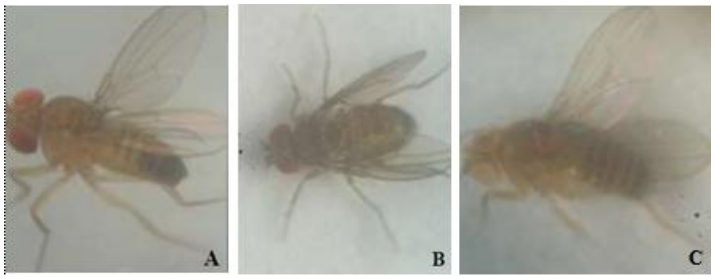

Figure 1. *D. melanogaster*:(A) Normal/*wildtype* strain; (B) *ebony body* strain; (C) and *white eyes* strain.

Mobile Phone Exposure

GSM mobile phone with provider using frequencies at 900/1800 MHz (3G) and HSDPA 2100 (4G) was used as a source of exposure (Figure 2a). The position of the mobile phone and flies crossbreeding bottles during this study is illustrated in Figure 2b. The crossbreeding performed were of homogamy types, which were:♂ N >< N ♀; ♂*e*><*e*♀; and ♂*w*><*w*♀.This study was conducted for five generations and the number of repetitionineach crossbreeding were four repetitions.

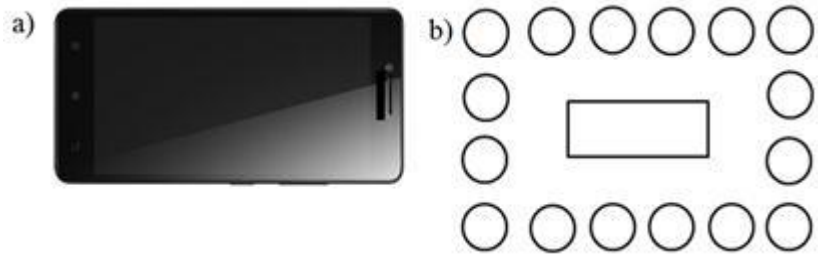

Figure 2. a) The mobile phone that was used in this study; b) the position of the mobile phone (rectangle) and the flies bottles (circle), the distance between the mobile phone with the bottles is ± 20 cm.

Data Collection and Statistical Methods

The number of adult filial males and females at each generation were recorded as the research data.The data were evaluated for statistical differences at a significance level of 0.05 by independent t-test if normality and homogeneity assumption were met and by Mann-Whitney U test if normality and/or homogeneity assumption were not met. The statistical analysis of the results were carried out using the IBM SPSS Statistics 22.0 programme.

RESULTS AND DISCUSION

The mobile phone exposure on *D. melanogaster* for five generations throughout the life cycle of flies has been done in this study. The number of adult males and femalesthat were recorded at each generation were the number of individuals that survived and were able to develop until adult stage. Table 1. shows the comparison between male and female adult filial number of *D. melanogaster* that exposed by mobile phone at each generationof each strain.

Based on Table 1., it can be seen although seems there were different number of males to females, but, after statistical test, there were no significant difference between the number of males and females at each generation of each strain. This is in agreement with the results obtained by Mirabolghasemi & Azarnia (2002) which their research is more focused on the frequency of abnormalities occurrence between males and females flies that exposed by EMF (Mirabolghasemi and Azarnia, 2002). In that report, the frequency of abnormalities flies on males and females did not differ significantly.

Table 1. The comparison between male and female adult filial number of *D. melanogaster* that exposed by mobile phone for five generations.

Strain	Generation	Male		Female		Sig.
		Total	Mean ± SD	Total	Mean ± SD	
N	1st	282	70.50 ± 8.699	252	63.00 ± 18.815	0.497
	2nd	209	52.25 ± 17.462	205	51.25 ± 26.361	0.952
	3rd	213	53.25 ± 26.550	217	54.25 ± 30.999	0.963
	4th	498	124.50 ± 15.416	491	122.75 ± 28.558	0.918
	5th	216	54.00 ± 11.225	232	58.00 ± 13.928	0.670
e	1st	164	41. 00 ± 5.715	154	38.5 ± 4.726	0.525
	2nd	264	66.00 ± 33.025	244	61.00 ± 38.009	0.849
	3rd	261	65.25 ± 12.527	261	65.25 ± 18.644	0.999
	4th	267	66.75 ± 28.004	295	73.75 ± 33.009	0.757
	5th	194	48.5 ± 6.658	197	49.25 ± 7.676	0.887
w	1st	261	65.25 ± 24.199	246	61.50 ± 19.070	0.343
	2nd	218	54.50 ± 8.387	214	53.50 ± 11.846	0.886
	3rd	183	45.75 ± 18.572	196	49.00 ± 25.232	0.686
	4th	249	62.25 ± 32.602	258	64.50 ± 34.771	0.928
	5th	155	38.75 ± 13.745	166	41.50 ± 13.379	0.784

In this study, the effect of gender on radiosensitivity are based on the sex ratio produced in each generation. Related to that, Moorhouse & Macdonald said sex ratio variation is commonly observed as a consequence of varying survival and value of offspring of different sexes (Moorhouse and Macdonald, 2005). So, the results from this present study indicate there are no varying survival between male and female on flies exposed by EMF. From this results, it can bee seen that, the radiosensitivity of male flies to EMF has the same level with female flies.

The odds of EMF did not seem to indicate that EMF may exert a gender-related influence on flies.But, this result is not consistent with some previous studies using other organisms. Papageorgiou, *et al* reported EMF may exert a gender-related influence on brain activity (Papageorgious *et al*., 2004). In another report, Moorhouse & MacDonald (2005) concluded the attachment of radio-collars to female causes changes in sex ratio on water voles (Moorhouse and Macdonald, 2005). Related to that, Alsbeih *et al.* (2016) suggested gender-related differences in radiosensitiviy may emanate from genetic and physiological dissimilarities between the two genders where generegulation and also hormonal factors may play important roles (Aslbeih *et al*., 2016). However, those previous studies used differentresearch subject and EMF source with this present study.

Related to the large standard deviation in this data (Table. 1), it is probable due to the large variation of offspring in each repetition.The large variation of offspring did not only occur inevery repetition, but also occur occur inevery strain and in generation. My previous report associate this condition with the concept that every individual has a different level of response to environmental stress (Fauzi *et al*., 2016). This is in agreement withBalmori that suggested "it is possible that each species and each individual, show different susceptibility to radiation, since vulnerability depens on genetic tendency, and physiologic and neurologic state of the irradiated organism (Balmori, 2009). Related to that, we recommend to the other researchers to conduct similar study, but with more repetitions, more strains, and more generations.

CONCLUSION

In this study, *D. melanogaster* were exposed by mobile phone for five generations. In the results, there were no significant difference (P>0.05) between the number of males to females at each generations of each strains.The finding of this study indicate that that EMF may not exert a gender-related influence.

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Composition of Sponges Associated with Mangrove in Tampora Beach, Situbondo, East Java

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ABSTRACT

This research began by identifying sponges samples from the west side of Tampora Beach, Situbondo, East Java, with an exploration area of 11.140 m^2. Sponge that has been collected by hand collecting method are documented and put into the sample bottle, fixed with 70% alcohol and preserved with 96% alcohol. The preserved sponge samples were cut into thin layer for spicules, longitudinal section, cross section and examined by the outer morphologycal character. The results showed that there are 4 sponges genera that lives on mangrove roots in Tampora Beach: Biemna Gray, 1867; Dysidea Johnston, 1842; Haliclona Grant, 1835; And Spheciospongia Marshall, 1892

Keywords: Identification, mangrove, spicules, sponges.

INTRODUCTION

Sponges (the phylum of Porifera) is the oldest multi-cellular invertebrate that has a body structure at the level of parazoa, because it has no true tissue, so the cells inside the sponge are totipotent and composed by spicules consisting calcium carbonate, silica and spongin fiber. The sponge can not move (sessile) and filters water for food (filter feeder) (Bergquist, 1978; Brusca *et al*., 2003).

Sponges divide into 4 major classes: Calcarea, Demospongia, Hexactinellida and Homoscleromorpha (Van Soest *et al*., 2012). Calcarea covering 8% of entire sponges species. Generally, this class have spicule composed by calcium carbonate and have one type of spicule. Demospongia covering 83% of entire sponges species. Generally, this class have spicule composed by silica, some species from this class also have spicules constructed from organic collagen fibre called spongin (Amir *et al*., 1996). Hexactinellida or glass sponge covering 8% of entire sponges species. Hexactinellida can be found in the ocean from depths of 200 to >6000 m. Generally, this class have spicules composed by silica. Homoscleromorpha covering 1% of entire sponges species. Generally, this class have spicules composed by silica (Van Soest *et al*., 2012). Sponges have the ability to adapt the physical- biotic components of water (Alcolado, 2003). Adaptation to physical- biotic component can be one of the factors that affect sponge growth rate in mangrove root. Mangrove ecosystems that grew along the sea shore have potential as habitat for variety of epibionts (Diaz *et al*., 2004; Farnsworth and Ellison, 1996). Epibiont is an organism living on the surface of another organism, including sponges (Nagelkerken *et al*. 2008). The mangrove root serve as habitat for sponges due to the lack of predators as well as the abundance of food sources (Diaz *et al*., 2004; Farnsworth and Ellison, 1996; Wulff, 2012). The mangrove ecosystem in the west part of Tampora Beach is grown by mangrove from *Rhizophora stylosa* species that have prop root system and *Sonneratia alba* which has pneumatophore root system. Both types of mangroves grow on mud substrate and dead coral.

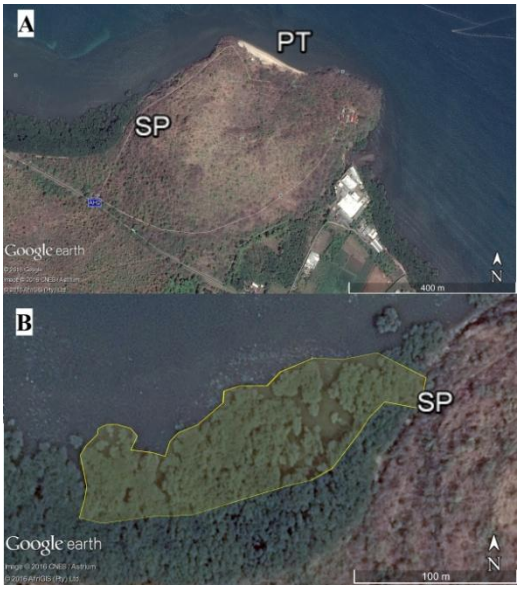

Figure 1. Sampling location in the west side of Tampora Beach (A) overall view of Tampora Beach (scale = 400 m) (B) sampling location (scale = 100 m).

MATERIAL AND METHOD

This research was conducted in the western part of Tampora Beach (7 ° 43'31.18 "S, 113 ° 38'43.07" E), in Banyuglugur subdistrict, Situbondo regency, East Java with exploration area of 11.140 m^2 and explored on August 2016 and January 2017. Tampora Beach geographical feature with substrate dominated with sand and rock cliffs, stretching from west side to east side. While the west part of Tampora Beach substrate is dominated with mud. Sample observations were conducted at Zoology Laboratory and Animal Engineering, Department of Biology, Institute Technology Sepuluh Nopember, Surabaya.

This research is divided into 3 stages: sampling stage, preservation stage, and sample processing stage in laboratory.

The preparation stage started by taking sponges from mangrove roots with hand collecting method with an exploration area of 11.140 m^2 at low tide. Then the sponge species were documented and put into the sample bottle. Sponges that have been put into the bottle then fixed and preserved with alcohol. Sponges have a body consisting of spicules. Researchers should avoid direct contact with the sponge by using of rubber gloves because spicules and biochemical compounds inside the sponge can cause irritation to the human skin. The processing stage is divided into two stages: spicule observation stage and cross-longitudinal section. The preserved sponge samples were cut and dissolved with bleach solution to obtain the spicules. Then the remaining sample is cut as thin as possible and to observe the ectosomal and choanosomal part of the sponge.

Identifying sponges use some data such as documentation and morphological descriptions. This data facilitates the researcher to identify the sponges that have been found. This research uses identification guide in the form of Guideline to the morphological species description for the Sponge Barcoding Database (SBD). The character of the SBD identification refers to Sponguide: Guide to Sponge Collection and Identification.

There are 10 identification characters for the sponge:

1. Growth form
2. Colour alive
3. Colour in ETOH
4. Oscules
5. Texture
6. Surface ornamentation
7. Choanosomal skeleton
8. Ectosomal skeleton
9. Megasclera
10. Microsclera

The morphological characters that were obtained then written based on 10 morphological characters of Guideline to the morphological species description for the Sponge Barcoding Database (SBD) and compared with the sponge characteristics from the Systema Porifera.

DISCUSSION

The mangrove ecosystem is a habitat for a wide variety of epibionts. Based on research in the mangrove ecosystem in the Caribbean, there are several organisms commonly found as epibionts in mangrove roots: sponges, algae, cnidaria and ascidia. Sponges composition in mangrove roots is very different from the composition of sponge species found in the sea. The composition of sponge species in mangrove roots is diverse, while in other roots can be controlled with only one type of sponge. This can be caused by extreme environmental physical-chemical factors: salinity, temperature and substrate type (Becking *et al*., 2013).

In the western part of Tampora Beach with exploration area of 11, 140 m^2, we found 10 species of sponges that come from four families: Biemnidae, Chalinidae, Clionaidae and Dysideidae. Sponge species that commonly found in this area is from Haliclona (Chalinidae). The mangrove species at the sampling site is dominated by *Rhizophora stylosa* and *Sonneratia alba*. Research on Bangka Island, North Sulawesi with an exploration area of 16.022 m^2 (Station 1 = 5.724 m^2; Station 2 = 10.298 m^2) found 10 species of sponges that are derived from seven families: Ancorinidae, Biemnidae, Chalinidae, Microcianidae, Niphatidae, Tethyidae and Thorectidae. Sponge species that commonly found in this area is from Haliclona (Chalinidae) and Amphimedon (Niphatidae). Mangrove species in Bangka Island is dominated by *Rhizophora* sp., While *Bruguiera spp.*, And *Sonneratia alba* is rarely found (Calcinai *et al*., 2016). Research in Kakaban and Maratua Island, Berau regency, East Kalimantan, with an exploration area of 199 million m^2, find 79 species of sponges from 27 families: Acarnidae, Ancorinidae, Biemnidae, Callyspongiidae, Chalinidae, Chondrillidae, Choelosphaeridae, Clathrinidae, Clionaidae, Darwinellidae, Dictyodendrillidae, Dictyonellidae, Dysideidae, Geodiidae, Halichondriidae, Heteroxyidae, Ianthellidae, Microcionidae, Niphatidae, Petrosiidae, Placospongiidae, Spirastrellidae, Suberitidae, Tedaniidae, Tetillidae, Tethyidae and Thorectidae. Sponges species that commonly found in this area is the Haliclona (Chalinidae). Mangrove species in Kakaban and Maratua dominated by *Avicennia marina*, *Sonneratia alba* and *Rhizophora mucronata* (Becking *et al*., 2013).

Mangrove ecosystems are generally found in the intertidal zones throughout tropical countries with sandy substrate, muddy substrate, and dead coral plains. Prop root and penumatohpores in mangroves that spread within and under the substrate are suitable habitats for various epibions, one of them invertebrates: sponges, anemones, polychaetes, bivalves, barnacles and ascidians (Nagelkerken *et al*., 2008; Calcinai *et al*., 2016).

Sponges in mangroves on the western part of Tampora Beach mostly located in the pneumatophore of *Sonneratia alba* (*Biemna* sp., *Dysidea* sp., *Haliclona* sp. "Beige branch", *Haliclona* sp. "Yellow", *Haliclona* sp. "White oscules", *Haliclona* sp "Purple", *Haliclona* sp. "Beige", *Haliclona* sp. "Brown" and *Spheciospongia* sp.). While other types of sponge are located in prop roots of *Rhizophora stylosa* (*Spheciospongia* sp.) And in tide pool (*Haliclona* sp. Velvet chimney, *Spongia* sp. And *Spheciospongia* sp.). Mangrove ecosystem in Indonesia that always inundated by water even though at the lowest tide, generally dominated with *Avicennia alba* and *Sonneratia alba* (Noor *et al*., 2012). While the area inundated during the high tide are dominated by *Rhizopora* sp. This statement corresponds to the location of the sample that found in the root of *Sonneratia alba*, because the pneumatophore of S. *alba* is still inundated by sea water at low tide which can become habitat for 4 sponge genera, whereas at prop root of R. *stylosa* there is only 1 genera of sponge. Different zone types in each mangrove habitat in the intertidal zone make the mangrove roots area lack of diversity of sponges. While subtidal areas that are not significantly affected by tides like seagrass beds, hard-edged beaches and coral reefs have more diverse type of sponges (Nagelkerken *et al*., 2008). The condition of sponges in mangrove exposed by strong currents in shallow waters is one of the factors causing low diversity of sponge in mangroves. The abundance of nutrients in the waters is often correlated with the abundance of sponges, if these nutrients are not mixed with pollutants present in waters, such as inorganic particles of industrial waste (Wulff, 2012).

Table 1. Comparison of species of sponges in mangrove roots.

Family	Species	Location found		
		TP	PB	BE
Acarnidae	*Acarnus* sp.			√
Ancorinidae	*Dercitus (Stoeba) bangkae*		√	
	Stelletta sp. *"icing"*			√
Biemnidae	*Biemna* sp.	√		
	Biemna fortis		√	√
Callyspongiidae	*Callyspongia diffusa*			√
Chalinidae	*Haliclona (Gellius)* sp.		√	
	Haliclona (Reniera) sp.1		√	
	Haliclona (Reniera) sp.2		√	
	Haliclona aff. *Baeri*			√
	Haliclona sp. *"beige"*	√		
	Haliclona sp. *"beige branch"*	√		
	Haliclona sp. *"blue crumble"*			√
	Haliclona sp. *"blue soft branch"*			√
	Haliclona sp. *"blue sticky"*			√
	Haliclona sp. *"blue white cloud"*			√
	Haliclona sp. *"blue branch"*			√
	Haliclona sp. *"bordeaux"*			√
	Haliclona sp. *"bordeaux soft"*			√
	Haliclona sp. *"brown"*	√		
	Haliclona sp. *"brown branch"*			√
	Haliclona sp. *"pale beige"*	√		√
	Haliclona sp. *"pink cloud"*			√
	Haliclona sp. *"purple"*	√		
	Haliclona sp. *"purple branch"*			√
	Haliclona sp. *"purple tough"*			√
	Haliclona sp. *"purple yellow"*			√
	Haliclona sp. *"soft spikes"*			√
	Haliclona sp. *"violet branch"*			√
	Haliclona sp. *"violet cloud"*			√
	Haliclona sp. *"violet soft"*			√
	Haliclona sp. *"violet tube"*			√
	Haliclona sp. *"violet vase"*			√
	Haliclona sp. *"white"*			√
	Haliclona sp. *"white cloud"*			√
	Haliclona sp. *"white extentions"*			√
	Haliclona sp. *"white oscule"*	√		
	Haliclona sp. *"yellow"*	√		√
	Haliclona sp. *"yellow branch"*			√
	Haliclona sp. *"yellow cloud"*			√
Chondrillidae	*Chondrilla australianensis*			√
Choelosphaeride	*Lissodendoryx isodictyalis*			√
Clathrinidae	*Clathrina* sp. *"white"*			√
Clionaidae	*Spheciospongia* sp.	√		√
Darwinellidae	*Darwinella* aff. *Gardineri*			√
	Dendrilla sp. *"black"*			√
	Dendrilla sp. *"pale"*			√
	Dendrilla sp. *"purple"*			√
Dictyodendrillide	*Acanthodendrilla* sp. *"burgandy"*			√
	Chelonaplysilla sp. *"gold"*			√
	Spongionella sp. *"green"*			√
	Spongionella sp. *"purple"*			√
Dictyonellidae	*Stylissa carteri*			√
	Stylissa massa			√
Dysideidae	*Dysidea* sp. *"black"*	√		
	Dysidea sp. *"blue"*			√
	Dysidea sp. *"tan"*			√
	Dysidea sp. *"white"*			√
	Lamellodysidea sp.			√
Geodiidae	*Geodia* sp. *"black"*			√
	Geodia sp. *"yellow"*			√
Halichondriidae	*Amorphinopsis* sp.			√
	Axinyssa mertoni			√
	Axinyssa sp. *"orange"*			√
	Halicondria sp. *"orange"*			√
	Hymeniacidon sp. *"yellow"*			√
Heteroxyidae	*Higginsia* mixta			√
	Higginsia sp. *"encrust"*			√
	Myrmekioderma granulata			√
Ianthellidae	*Hexadella* aff. *indica*			√
Microcionidae	*Antho ridleyi*			√
	Clathria (Microciona) sp.		√	
Niphatidae	*Amphimedon* sp.1		√	
	Amphimedon sp.2		√	
	Amphimedon sp. *"blue-green"*			√
	Amphimedon queenslandica			√
Petrosiidae	*Neopetrosia* sp. *"blue"*			√
Placospongiidae	*Placospongia* spp.			√
Spirastrellidae	*Spirastrella* aff. *Decumbens*			√
	Spirastrella aff. *solida*			√
	Spirastrella sp. *"orange"*			√
Suberitidae	*Suberites diversicolor*			√
Tedaniidae	*Tedania diraphis*			√
Tetillidae	*Cinachyrella* spp.			√
	Paratetilla bacca			√
Tethyidae	*Tethya* aff. *Bulae*			√
	Tethya aff. *coccinae*			√
	Tethya aff. *sechellensis*			√
	Tethytimea aff. *tylota*			√
	Timea sp.		√	
	Timea sp. *"yellow"*			√
Thorectidae	*Cacospongia* sp. *"shiny"*			√
	Hyrtios communis		√	
	Scalarispongia sp.		√	
	Semitaspongia sp. *"black"*			√
	Semitaspongia sp. *"beige"*			√
Total Species found		**10**	**10**	**79**
Total Family		**4**	**7**	**27**

Tampora – East Java (**TP**); Bangka Island – Nort Sulawesi (**PB**) (Calcinai *et al*., 2016); Berau – East Kalimantan (**BE**) (Becking *et al*., 2013).

Tidal pool formed by erosion of dead coral rock in intertidal area by ocean current and trapped water in the gap/basin that have beem formed. Tidal pools are productive microhabitats because there are a variety of organisms inside such as; microalgae, invertebrates, and fish. Tidal pools are common in the intertidal zone. Each tidal pool in the same intertidal zone can have different diversity of different organism types (White *et al*., 2015). This makes some species of sponges have physiological and morphological adaptation properties that can make sponge species tolerant to the extreme physical-chemical factor alteration at the lowest tide, in order to survive (Wulff, 2012). Most sponge-related research as epibionts on mangroves in the world comes from the Caribbean islands. The average mangrove sponge in Indonesia is small and has a dull color (Becking *et al*., 2013; Calcinai *et al*., 2016). This corresponds to the species of sponge found in Tampora such as the Biemna and Haliclona that have dull colors. While sponges in the mangrove in the Caribbean island has bright colors, large size and massive form of growth. The Caribbean waters have a tidal range no more than 0.5 m with 4 hours tidal time (Anonim, 2016). While the tidal range on Tampora Beach reaches 1.1 m with 5 hours tidal time (Anonim, 2016). Some types of sponges can survive if exposed to the sun directly by burrowing some of the body into the substrate of mud to hold water that trapped within the body for several hours (Rützler, 1964). This can be seen in *Spheciospongia* sp. and *Biemna* sp. Which part of its body gets into the muddy substrate. In addition, direct sun exposure also affects the growth of the sponge. If the sponge is too long exposed to the sun's heat, then the water that has been trapped in the body of the sponge will evaporate. This makes the sponge unable to filtrate the previously collected water in order to obtain organic compounds as a source of energy. So most sponges prefer non-sun-induced habitats, such as shady gaps in rocks, mangrove canopies and overgrown seagrass areas (Anonim, 2016). The sponges community of mangroves in the Caribbean is different from the sponges communities on the coral reefs, and some species of sponges in the Caribbean have adaptability to extreme salinity, temperature and sediment conditions (Calcinai *et al*., 2016).

The number of sponges species in mangrove is limited. This happen because of factors such as: duration of exposure to air (desiccation / drying) during low tide, wave movement, temperature and salinity [12]. Differences in tolerance between species of sponge when exposed to air cause a vertical sponge zonation in the *Rhizophora mangle* prop root. The most resistant species of sponge will be at the top of the R. *mangle* prop root adjacent to the highest tide (Rützler, 1964).

CONCLUSION

From the research conducted it is known that in the intertidal zone in Tampora Beach, we found 4 sponge genera associated with the mangrove roots: *Biemna* sp., *Dysidea* sp., *Haliclona* sp. and *Spheciospongia* sp. The next research of sponge in mangrove should be explored in the eastern part of Tampora (Besuki, Situbondo, to Baluran National Park) and the western part of Tampora (Beejay Bakau Resort, Probolinggo).

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Plankton Diversity Index in Estuary of Musi River to Determinate the Quality of Waters as Habitat of Fishes

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ABSTRACT

The Estuary of Musi River waters had many functions, namely as habitat of fishes and Shipping Pathways for fisherman and others importance enter to Palembang harbors. Existence plankton community as diversity and it's densities so very important to suply much nutrition for foodstuff the juvenile of fishes in estuary of Sungsang. Acording to my research some years to prove that at waters of wetland like Musi River always acumulate much organic matter eventhough from domestic or natural material. Much of natural process works in the surface level of rivers by much plankton organisms. According to my research in these years can expose the relation between the populations of several fishes and biodiversity and population of plankton diversities. Base of my research, the population of plankton in downstream of Musi River estuary in Sungsang regional so depleted and the diversity of plankton communities also mediately because any factors which limiting growth and development each species of plankton communities. These conditions determinate the population of fishes become lower from last time upto recent time. The probility of these cases pertaining to some physical and chemical factors which give effect to quality of waters of Musi River estuary like in Sungsang Delta. The population of plankton communities at these research 53 up 76 individu/ liter. The diversity index of plankton communities were 2.37 upto 3,48. The upstream of sungsang estuary was very important as habitat for fishes in the ecosystem of Sungsang estuary, related to abundance and more rich plankton when compared to central and downstream of Sungsang estuary. Plankton Diversity Index in Estuary of Musi River can to Determinate the quality of waters as the Quality of Waters as Habitat of Fishes. The species of fishes were 9 species which found during research in 20th August 2016.

Keywords: Estuary, Musi rivers, plankton communities, fishes.

INTRODUCTION

Much of waters of all streams and rivers from Musi River areas eventually drain into the sea, by direct and indirectly. The place where this fresh waterfrom Musi Rivers joins to the salt water in Sumatera Straits is called an estuary, and this location generally was mentioned as Muara Sungsang. More precisely, these estuaries are semienclosed parts of coastal ocean where the seawater is diluted and partially mixed with water coming from land and the edge of waters was growed by mangrove vegetation. According to Smith (1986) that estuaries differ in size, shape, and volume of water flow, all influenced by geology of the region in which they occur. The Muara Sungsang as estuary was made of delta which mentioned as Sungsang delta. This area so important for inter and outer big upto little ship as gate or pathways to Palembang City of South Sumatera. Much activities a long side of Musi River from upstream so far a way until down stream near estuary had contribute much material to water body and causing decrease of water quality. The activities who made decrease of waters in Musi Rivers namely industries and pabrics like Remiling or Crumb Rubber, Petrochemical, Palm oil, Pulp papers and other activities

like Traditional markets, Hospitals and domestic waste a long of rivers. Other waste so important namely plantation of rubber and palm so extensive in south sumatera. The estuary of Sungsang so important for waters pathway and natural resources. As natural resources, estuary of Sungsang was the habitat for macro and microorganism in aquatic ecosystem. As a aquatic ecosystem that estuary of Sungsang so important for product much fishes which had been contributing to fishermen living every day. The microorganism which important in aquatic ecosystem was plankton. The macroorganism which main important in aquatic ecosystem was nekton namely fishes. The plankton and nekton were two group of organism so important in food chain in aquatic ecosystem like in Sungsang area.

The plankton comprises all those aquatic organisms which drift passively or whose powers of locomotion are insufficient to enable them to move contrary to the motion of their inhabitat water mass (Barnes and Mann, 1980: 6). Of course, this is not to suggest that plankton are necessarily incapable of moving within a given a water mass; motile spesies may move vertically in a laterally-flowing current in much the same way that one may move up and down stairs in a double-decker bus without affecting one's movement relative to ground (Barnes and Mann, 1980: 6). The movement of plankton depend to nutrition supply and hence they reproduce very quickly in surface level of those little streams and many pools when the nutrition for them so much in waters. In generally according to trophic level, plankton comprise of phytoplankton and zooplankton. In the communities of plankton in water ecosystem at the time and space given that phytoplankton more little of cell sizes than zooplankton.

Phytoplanktons play an important role in aquatic ecosystems, both in freshwater and in marine environment. They are the primary producer organisms, therefore, supporting zooplanktons, fishes and other members of aquatic fauna. Thus, they are placed at the base of the trophic strata or at the bottom of the aquatic food web. Phytoplanktons also play a major role in global carbon dioxide fixation. Phytoplanktons also maintain the oxygen level of the water body, which is designated as dissolved oxygen or DO (Pal and Avik, 2014:23).

In wetland ecosystem like estuary of Sungsang, that phytoplanktons are one of the fundamental players of physical, chemical and biological processes that characterize the wetland ecosystems. They mostly act as primary producer, therefore placed in the wetland food web (Pal and Avik, 2014): 29. Physical factors affecting wetland ecosystem are light and temperature. Light penetration in water column of shallow wetlands is affected greatly by winddriven sediment resuspension which also increases the productivity of shallow wetlands (Klarer and Millie 1992 *in* Pal and Avik, 2014). The bottom sediments are also subjected to regular disturbances by the benthivorous fishes like: walking catfish (*Plotosus canius*). In wetland ecosystem differences in temperature with depth are minor, as the wetlands are shallow and the wind-driven mixing minimizes the temperature gradient of wetland water column. Sometimes the thick algal mats or floating mat of duckweed may increase the temperature variation in water column reducing the temperature (Pal and Avik, 2014:30). The zooplankton is a general term for pelagic animals like protozoa, copepods, ostracods, cladocera, rotifer and other microfauna which are unable to maintain their position by swimming against the physical movement of water. The life cycles of the zooplankton are extremely varied and for specific groups of organisms that consist of invertebrate. Phytoplankton. Existence of zooplankton very important to life of fish juveniles in aquatic ecosystem and on the contrary existence of phytoplankton also very important to life of zooplankton communities in aquatic ecosystem. The little rivers in South Sumatra need be conservated persistently because they play a role important to contribute microhabitat as nursery ground for much freshwater fishes (Sagala, 2014).

Plankton diversity Index can be used for measuring or determinating the quality of water as habitat of fishes. The index diversity of plankton community would point out the stability of plankton living in habitat. Stability of plankton show the condition of abundance or densities of each population of species and the richeness of plankton community. According Sagala (2014), Indices values of diversity can be categorize as follows: Indices diversity as big as: ≤ 1: can be said that the communties of organism very non stable in the ecosystem becouse any problems of physical, chemical and biological factors interference some communities of organism. If indices of diversity as big as > 1 - < 2,0: can be estimate that the communities of organism non stable in the ecosystem by some factors of biological, physical and chemical. When the indices diversity as big as: > 2,0 – 3,0 mean that the

communties of organism adequate stable in the ecosystem. Furthermore, if the indices diversity as big as: > 3,0 mean that the communties of organism very stable in the ecosystem.

The diversity index value can be interrelated to existence and population of fishes in habitat. The condition of plankton community in estuary of Sungsang was affected by the quality of water drain from upstream and buffer zone around like mangrove. The mangrove in location enough naturally showed from dense of vegetation and the old of vegetation.

MATERIALS AND METHODS EXPERIMENTAL

This research had been made in Banyuasin Region of South Sumatra Provincy on wetland areas, around of Delta of Sungsang. Some materials which used in the fields were plankton net no. 25, benthic net, cold box, camera (Canon Merk), pH Stick (Merck), Column Thermometer, pail vol. 10 liter and flacon bottles. The substrates of sediment and water samples from fields carryout to laboratorium for analysing the chemical contents. The physical features directly were measured in the swamps like temperature and transparency. Sample of plankton taken with using plankton net as much as 50 liter and the precipitatate take in flacon, and then be given 4 drops formalin. Furtheremore, the sample of plankton inspected under the microscope in 150 X and 400 X magnitude and than recorded as data.

Diversity index of communities: $H = - \sum pi \ln pi$; $pi = Ni/N$

The symbol H = Shannon index of general diversity. In here as Diversity Index of Plankton Community (Shannon index); pi = Importance probability for each species = ni/N; Ni = importance value for each spesies; N = total importance value (Odum, 1971: 144).

The fishes which caught by fishermen around location during sampling plankton in location recorded and than identified in laboratory.

RESULTS AND DISCUSSION

Acording to plankton analysis at three sampling location based to direction of water current in Sungsang Estuary, point out that diversity index of plankton community were different enough for three location. Diversity index of plankton community on the upstream of estuary, apparently higest compared with central and down stream of Sungsang Estuary (Table 1). The lowest of diversity index of plankton community found on central of Sungsang Estuary. This condition at the central of Sungsang Estuary had been done mixing current from inland waters and sea waters and than plankton population eliminated or probably diverse around waters. This situation effect the population of plankton tobe descended. Fishes in estuary lookfor the sites which much or blooming plankton maybe foodstuff directly or indirectly. As inderectly, the plankton population tobe food chain for little fishes, and than this little fishes tobe foodstuff fo the big fishes. Apparently, the high population of plankton can be found in the upstream of Sungsang estuary. This condition had proved that there were root related between diversity index of plankton with the quality of waters as habitat for fishes.

The complete of plankton analysis can be found in Tabel 1. The population plankton community on all sites were 53 upto 76 individu/liter of water sample. This value can be said the medium abundance, namely, 50 upto 100 individu/liter. Acording to level of trophic, the waters of study area can be said the mesotrophic waters.

The richness of plankton spesies on the upstream were highest were compared to other sites of sampling station. The 44 species which can be found in the upstream showed that the habitat of fishes in those place better than others. Such as, on the upstream of sungsang estuary made up as important habitat for fishes in the ecosystem of Sungsang estuary. Overall , the richness of plankton community in estuary ecosystem of Sungsang area were 63 species, this condition include moderate, namely between 50 upto 100 species.

Tabel 1. Analysis results of plankton population on three sampling location of Sungsang estuary.

No.	Group of Plankton and Species	Population: Individual x liter $^{-1}$		
		Sampling location:		
		P1	P2	P3
	A. PHYTOPLANKTON:			
	I. Cyanophyceae:			
1.	*Lyngbya limnotica*	-	-	1
2.	*Nodularia spumigena*		-	-
3.	*Oscillatoria curviceps*		-	1
4.	*Oscillatoria lacustris*		-	-
5.	*Oscillatoria limosa*	-	1	-
6.	*Phormidium tenue*		1	3
	II. Chlorophyceae:			
1.	*Chaetophora elegans*		1	-
2.	*Microspora tumidula*		4	-
3.	*Oedogodium varians*		1	-
	III. Desmidiaceae:			
1.	*Closterium libellura*		-	1
2.	*Gonatozyga moniliformis*		-	-
	IV. Diatomae:			
1.	*Amphipleura pellucida*		1	-
2.	*Bacillaria paradoxa*		2	-
3.	*Cyclotella operculata*		-	-
4.	*Cyclotella stelligera*		-	1
5.	*Coscinodiscus oculus*		3	7
6.	*Detonula schroederi*		5	-
7.	*Diatoma elongatum*		3	-
8.	*Diatoma vulgare*		-	-
9.	*Eunotia arcus*		1	-
10.	*Melosira varians*		8	1
11.	*Nitzschia linearis*		2	3
12.	*Rhizosolenis alata*		1	-
13.	*Stephanodiscus corcoensis*		7	2
14.	*Surirella tenera*		1	-
15.	*Tabellaria fenestrata*		3	-
	B. ZOOPLANKTON			
	I. Flagellata:			
1.	*Carteria crucifera*		-	-
2.	*Carteria globosa*		1	-
3.	*Ceratium tripos*		-	-
4.	*Euglena deses*		2	-
5.	*Phacus pleronectes*		-	-
6.	*Monas vivipara*		-	1
7.	*Oicomonas socialis*		7	2
8.	*Thylacomonas compressa*		1	-
9.	*Trachelomonas abrupt*		1	-
10.	*Trachelomonas cervicula*		2	2
11.	*Trachelomonas curta*		1	-

12.	*Trachelomonas oblonga*	4	1	-
	II. Rhizopoda:			
1.	*Astramoeba radiosa*	1	1	5
2.	*Centropyxis ecornis*	1	-	1
3.	*Ditrema flavum*	-	1	-
4.	*Difflugia urceolata*	1	-	-
5.	*Nebela dentistoma*	-	-	1
6.	*Nebela militaris*	-	-	1
	III. Rotifera:			
1.	*Brachionus falcatus*	-	-	1
2.	*Kellicottia longispina*	-	-	1
3.	*Monostyla* sp.	-	-	1
4.	*Rotaria rotatoria*	-	1	-
	IV. Cladocera:			
1.	*Ceriodaphnia* sp	4	-	1
2.	*Chydorus ovalis*	-	-	1
3.	*Simocephalus vetulus*	-	-	2
	V. Ostracoda:			
1.	*Cypricercus reticulates*	-	-	2
2.	*Cypridopsis aculeate*	1	-	2
3.	*Cypris pubera*	-	-	1
	VI. Copepoda:			
1.	*Calanus finmarchicus*	-	3	3
2.	*Cyclops magnus*	1	1	1
3.	*Cyclops vernalis*	-	-	3
4.	*Diaptomus leptopus*	-	-	1
5.	*Diaptomus* sp.	-	2	2
6.	*Eurytemora affinis*	1	-	-
7.	*Harpacticus chelifer*	1	-	1
8.	*Cyclops* sp. (Stadium: Nauplius)	-	19	4
	VII. Mollusca:			
1.	*Corbicula sp*.(larva: glochidium)	1	-	2

Parameter:

1.	Population of Plankton Community:	75	53	76
2.	Population of Phytoplankton Community:	44	19	32
3.	Population of Zooplankton Community:	31	34	44
4.	Richness of Plankton Species:	33	19	42
5.	Richness of Phytoplankton Species:	16	8	15
6.	Richness of Zooplankton Species:	17	11	27
7.	Diversity index of Plankton Community:	3.19	2.37	3.48
8.	Dominance index of Plankton Community:	0.053	0.160	0.041

Primary Data: 20th, September 2016.

Explanation of sampling location: P1 : Downstream of Sungsang Estuary (S: 02 º 20’24,8”; E: 104º55’33,4”); P2 : Central of Sungsang Estuary (S: 02 º 21’51,5”; E: 104º54’15,7”); P3: Upstream of Sungsang Estuary (S: 02 º 21’52”; E: 104º54’45”).

The results of plankton analysis can be showed on Tabel 1, that the highest diversity index of lankton community can be found in the upstream of Sungsang estuary, and this condition so related to the qualithy of waters as habitat of fishes. Acording to data of population of fishes (Tabel 3), that the highest of fishes population root related to diversity index of plankton community (Tabel 1). And this condition also related to some parameter as dissolved of oxygen and water transparency and others parameter.

Tabel 2. Estimation of Fishes which caught by Fisherman along Sungsang Estuary when plankton sampling had been taken at same time in 20th September 2016.

No.	**Species**	**Estimation of Population**		
		P1	P2	P3
1.	*Sciaena macropterus*	+	-	++++
2.	*Batrachocephalus mino*	+	+	+++
3.	*Cynaglossus lingua*	-	+	++
4.	*Plotosus caninus*	+	-	-
5.	*Parastromateus niger*	+	+	+
6.	*Cyclocheilichthys enoplos*	-	-	+++
7.	*Synaptura commersoniana*	-	-	+++
8.	*Chela hypothalmus*	-	-	+++
9.	*Pangasius micronemus*	-	-	++

Explanation: -: absent/not found; +: little, less of 10 ten individuals; ++: > 10 upto 20 invidual;
+++: > 20 upto 30 individual; ++++: > 30 invidual. Tool for finding sample namely: gillnet
Primary Data: 20th, September 2016.
Explanation of sampling location: P1 : Downstream of Sungsang Estuary (S: 02 º 20'24,8"; E: 104º55'33,4"); P2 : Central of Sungsang Estuary (S: 02 º 21'51,5"; E: 104º54'15,7"); P3: Upstream of Sungsang Estuary (S: 02 º 21'52"; E: 104º54'45")

According to Tabel 2, apparently the highest population of fishes in Sungsang estuary can be found in upstream Sungsang area on mouth of Musi River. This is reason why the fishermen take many their gill net around of sites. All of species of fishes which catched by gill net of fishermen were same of time with taking the sample of plankton. The fish that abundance if campared to others species was spesies, *Sciaena macropterus* (gulamah, local name), namely more 30 individu catched on the time. The species of *Cyclocheilichthys enoplos* (lumajang, local name), *Batrachocephalus mino* (utik, local name), *Synaptura commersoniana* (sebelah, local name) and *Chela hypothalmus* (janggut, local name) were more abundance. And than, *Cynaglossus lingua* (lidah, local name) and *Pangasius micronemus* (patin, local name) were less abundance. And last, *Parastromateus niger* (bawal hitam, local name), was scarce or no abundance.

Tabel 3. The results of chemical and physical analysis of Sungsang estuary waters.

No.	**Parameters**	**Locations:**		
A.	Physical Parameters:	P1	P2	P3
1.	Temperature (ºC):	30	30	30
2.	Light penetration/ transparency (cm):	10	19	44
3.	Electrical conductivity:	603	236	499
4.	Turbidity:	9	11	50
5.	TSS (total suspended solid):	25.75	16.5	17.4
B.	Chemical Parameters:			
1.	pH:	6.53	6.47	6.49
2.	Total ammonia (mg/l):	0.16	2.28	8.4
3.	PO_4 (mg/l)	0,51	0,50	0,59
4.	Sulfide (mg/:	0.025	0.031	0.099

5.	Nitrate (mg/l):	0,6	1.0	1.3
6.	COD (Chemical Oxygen Demand) (mg/l):	14.73	9.91	21.18
7.	BOD (Biological Oxygen Demand) (mg/l):	2.34	1.83	4.10
8.	DO (Dissolved Oxygen):	6.63	5.61	7.64
9.	Fad and oil (mg/l)	3.2	3.4	3.8

Primary Data: 20th, September 2016.
Explanation of sampling location: P1 : Downstream of Sungsang Estuary (S: 02 º 20'24,8"; E: 104º55'33,4"); P2 : Central of Sungsang Estuary (S: 02 º 21'51,5"; E: 104º54'15,7"); P3: Upstream of Sungsang Estuary (S: 02 º 21'52"; E: 104º54'45").

Based to Tabel 3, more many the parameters of water which had measured relatively same one and another, but some parameters show a little difference. Some important parameters that showing the difference were DO (dissolved oxygen) and water transparency. DO, as parameter very important to activity of metabolisme of organisms in water ecosystem, play a role to respirate of aquatic organism like fishes, plankton and others. The water transparency also very important to activity of photosynthetisis in aquatic column by phytoplankton which produce organic matter and dissolved oxygen.

CONCLUSION

- The upstream of sungsang estuary was very important as habitat for fishes in the ecosystem of Sungsang estuary, related to abundance and more rich plankton if compared to central and downstream of Sungsang estuary.
- Plankton Diversity Index in Estuary of Musi River can to Determinate the quality of waters as the Quality of Waters as Habitat of Fishes.

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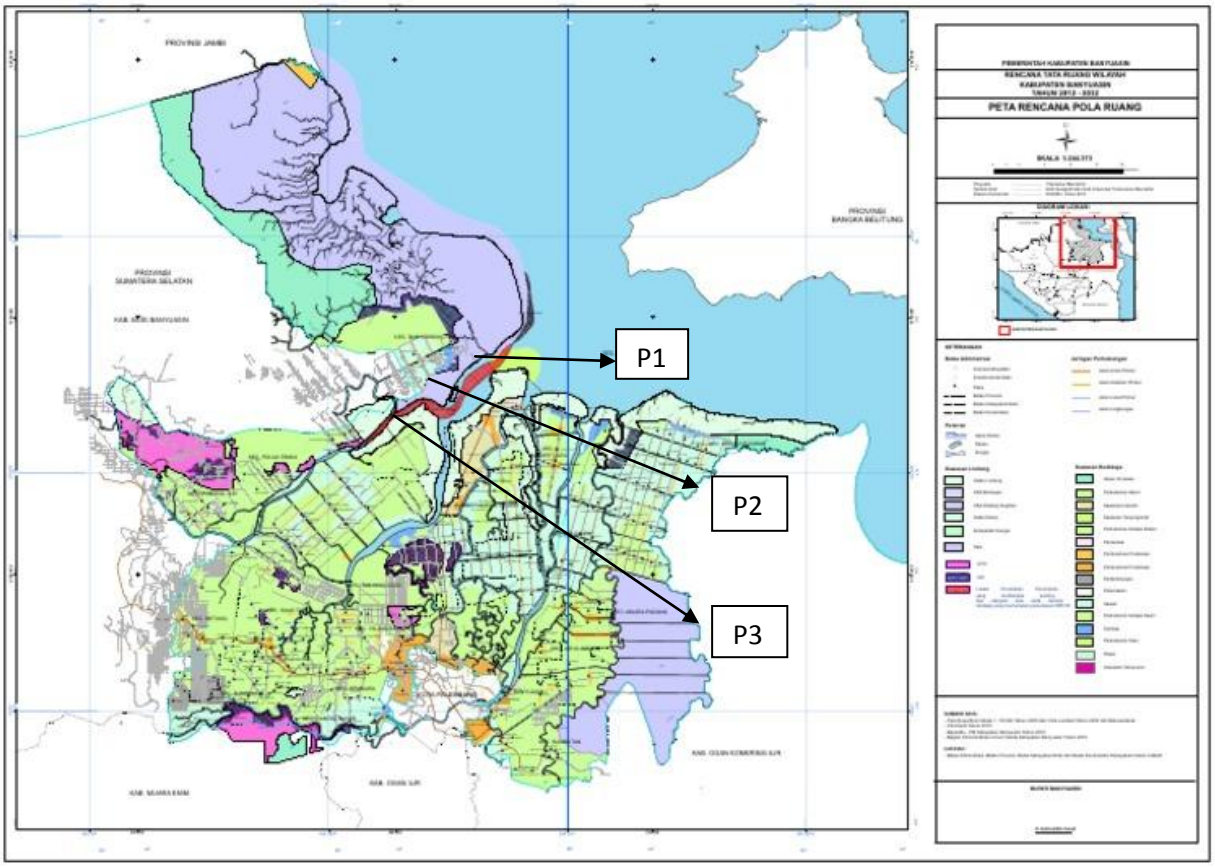

Figure 1. The sites of Sampling Location (August, 20th 2016).
Explanation of sampling location: P1 : Downstream of Sungsang Estuary (S: 02 º 20'24,8"; E: 104º55'33,4"); P2 : Central of Sungsang Estuary (S: 02 º 21'51,5"; E: 104º54'15,7"); P3: Upstream of Sungsang Estuary (S: 02 º 21'52"; E: 104º54'45").

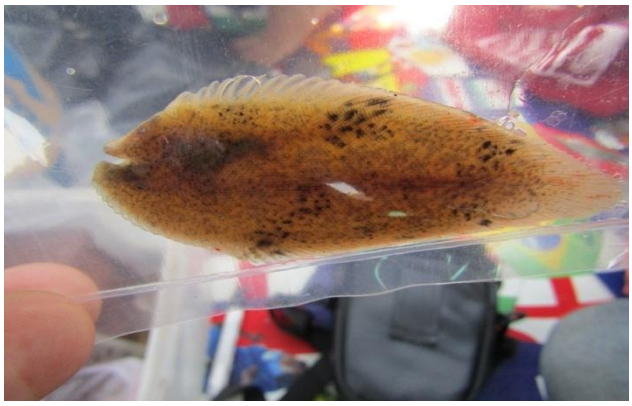

Figure 2. *Cynaglossus lingua* (lidah, local name).

Figure 3. *Cyclocheilichthys enoplos* (lumajang, local name).

Figure 4. *Synaptura commersoniana* (sebelah, local name).

Figure 5. Some kind of fishes who catched by fishermen, 20th August, 2016. In figure appear: *Sciaena macropterus* (gulamah, local name) and *Synaptura commersoniana* (sebelah, local name).

Figure 6. *Pangasius micronemus* (patin, local name).

Grey Water Treatment Using Plant of *Scirpus grossus*in Biofiltration System

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ABSTRACT

Since municipal wastewater management relies on communal wastewater treatment plant targeting only black water treatment processes, an opportunity to treat grey water and conserve it as one of water resources has become an emerging concern. Generally in Indonesia, grey water are discharged in to drainage system. Biofiltration systems were designed to reduce concentrations of pollutants. Plant species used in biofiltration systems have commonly been selected based on their potential for high uptake of some pollutants, such nitogen and phosphate. *Scirpus grossus*is an aquatic species with a high growth rate and has the ability to degrade pollutants. The aim of this research were to determine of some pollutant parameters removal in grey water using plant of *Scirpus grossus* in biofiltration system. The reactor of biofiltration was made of acryclicwith dimensions of 14 cm for diameter and 50 cm (*H*). Soil, sand and gravel were used for media in biofiltration reactor. The reactor biofiltration was operated in batch system. There were four reactors, namely biofiltration reactor A - with sand and gravel without plant, biofiltration reactor B - with soil and gravel without plant, biofiltration reactor C - with sand, gravel, and plant; and biofiltration reactor D - with soil, gravel, and plant. Based on results, the highest percentage removal of TSS, COD, and TN occured in reactor D, sand, gravel, and *S. grossus*, with removal were 88.2%, 88% and 77.9%, respectively. It indicates that plantof *Scirpus grossus* has a role to removeTSS, COD,and Total Nitrogen from grey water.

Keywords: biofiltration, grey water, pollutants, *Scirpusgrossus*

INTRODUCTION

Wastewater treatment plants, which were mostly constructed in cities, have served on treating domestic wastewater for decades. However, nowadays, as ecological sensitive areas,rural regions should be paid more attention and rural wastewater should be treated on-site in order to be kept away from ground water or natural aquatic environment. Soil filtration, as an environmentally friendly on-site process, has been widely applied andpromoted for years in many developing countries, especially inthose rural areaswhere water pollution is crucial and severe (Yuan *et al*., 2013).

Constructed wetland, characterized by simple maintenance, energy-saving and high efficiency, has become one of the most common soil treatments (Vymazal and Ezinová, 2015). However, large land occupation, seasonal changes, vegetation diseasesand insect pests were main obstacles for application of constructed wetland (JDíaz *et al.,* 2010). To shrink space occupationand cut energy cost, small-scale biofilter packed with highefficient water-permeable media instead of conventional soil, was supposed to be an appropriate solution for rural wastewater on-site treatment (Wang *et al.,* 2010; Guan *et al*., 2012; Li *et al*., 2012; Pan *et al.,* 2013).

Domestic wastewater usually contains components of organics, nitrogen and phosphorus. Efficient removing most of contaminants simultaneously or in turn within one-stage seemed interesting and convenient for wastewater treatment, especially for nitrogen removal, conversion mechanism of $NH_4^+ \rightarrow NO_3^- \rightarrow N_2$ was always

essential for Total Nitrogen (TN) removal. Therefore, an integration of aerobic digestion/nitrification and anaerobic denitrification should be realized to promote soil biofilter system.

Biofiltration systems are storm water control measures specifically designed to reduce concentrations of pollutants discharged into urban water ways, including: N, P, and heavy metals (Davis et al., 2009; Fletcher et al., 2014). They consist of anengineered profile of filter media (primarily sand) planted with vegetation that has high nutrient uptake potential (Hatt et al., 2008); Hsieh and Davis, 2005).

Plant species used in biofiltration systems have commonly been selected based on their potential for high uptake of dissolved N and P pollutants, although a range of other local drivers of species selection have also been considered (Hunt et al., 2015). Plant of *S. grossus*is commonly found in tropical and temperate regions such South East Asia, i.e. Indonesia, Malaysia, India, South China, and tropical partin Australia. *S. grossus*is emergent plants are dominate in wetlands, shallow lakes and streams. *S. grossus* is an aquatic species with a high growth rate and has the ability to degrade contaminants. It has sharp to soft stems, triangular leaves, obvious leaf blades, inflorescences always on the stem tips, seen as tight clusters or spreading open with the leaves resembling stems (Kostermans et al., 1987).

The aim of the research was to determine of TSS (*Total Suspended Solid*), COD (*Chemical Oxygen Demand*), and Total nitrogen removal in grey water using biofiltration system with plant of *Scirpus grossus*. Second aim was to determine effect of usage different media, i.e. sand and soil at ITS campus in biofiltration system. Besides that, to monitor some parameters such pH and temperature during operation of biofiltration system.

MATERIALS AND METHODS

Artificial Grey Water

Artificial grey water was used in the experiment to assure consistency and repeatability. The composition was similar to the formulation reported by Travis et al. (2010). A volume of 500 L of artificial grey water was prepared before biofiltration run according to the proportions shown in Table 1.

Table 1 Artificial grey water formulation.

Componen	Amount
Water	500 L
Laundry powder	40 g
Bar soap	50 g
Vegetable oil	7.5 g
Raw dining hall/kitchen effluent	300 mL

Biofiltration Reactor for Laboratory Scale

The biofiltrationreactor units are made of acrylic, with dimensions of 14 cm for diameter and 50 cm (*H*). Pipe are made of PVC with diameter of ¾ inchi. Figure 1 describes the design of biofiltration system reactor. Soil, sand and gravel with diameter of 1 cm and 2 cm were used for media in biofiltration reactor. The depth of both the medium and fine gravel layers were 10 cm. Meanwhile the depth of soil or sand was 20 cm.The reactor biofiltration was operated in batch system. There were four reactors, namely biofiltration reactor A - with sand and gravel without plant, biofiltration reactor B - with soil and gravel without plant, biofiltration reactor C - with sand, gravel, and plant of *S. grossus* (Sg); and biofiltration reactor D - with soil, gravel, and plant of *S. grossus* (Sg).

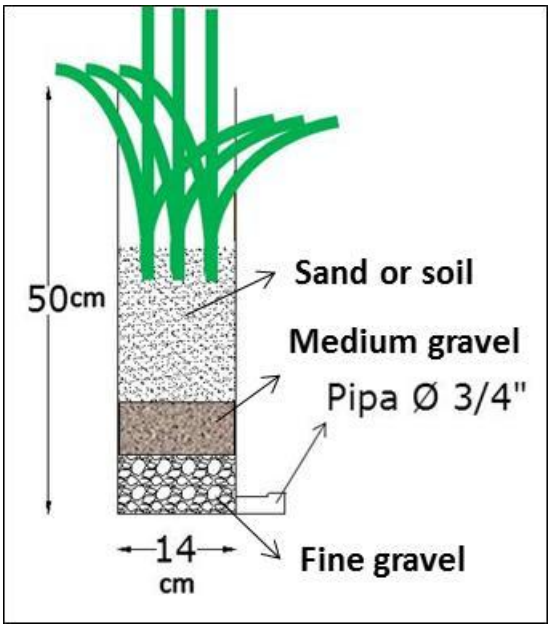

Figure 1. Design of biofiltration reactor.

Analysis of Parameters

The analyzed parameters in this research wereTSS, COD,and Total nitrogen in grey water, while parameters were be monitored are pH and temperature. All parameters were be analysed in Department of Environmental Enginering, Institut Teknologi Sepuluh Nopember (ITS). The methods and instruments of this research were listed in Table 2.

Table 2. Parameters to be analysed throughout the laboratory study.

Parameter	Method	Reference
TSS	Gravimetric analysis	Standard Methods for Water and Wastewater, APHA
COD	Closed Reflux and Titrimetric Method	
Total Nitrogen	Persulfate digestion method followed by Spectrophotometric method	
pH	Samples will be tested using a pH meter	
Temperature	Samples will be tested using a termometer	

RESULTS AND DISCUSSION

Monitoring of pH and Temperature

Based on Figure 2, in general, the results showed that the moisture ranged from 7.3 to 8.15. The average pH ranged from7.2 – 0.1 to 8.0 – 0.2, in the normal range for plant growth[15]. Figure 3 showed temperature during biofiltration test. In general, the results show that the temperature values 24 ◦C through-out the 7 days; this is normal for a tropical region.

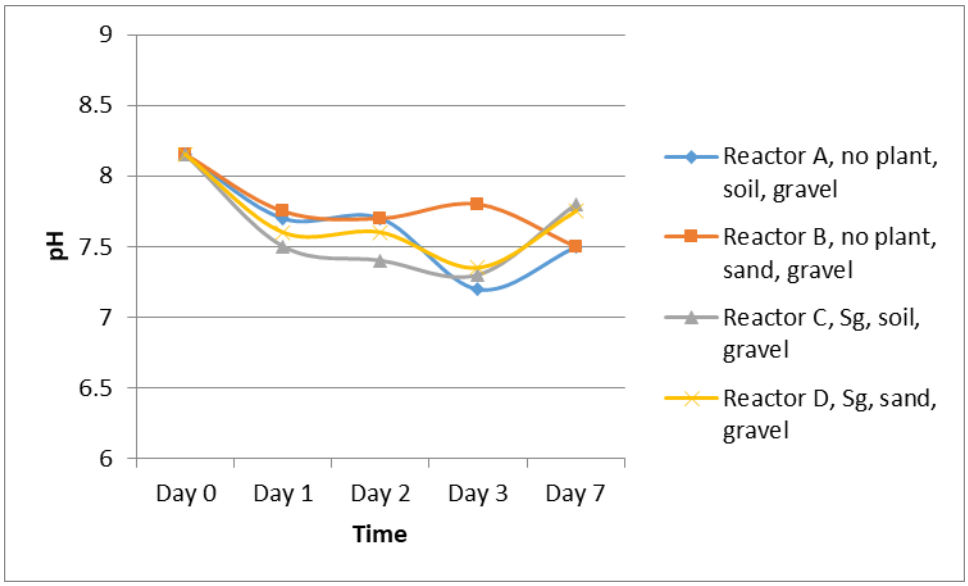

Figure 2. pH of every type of reactors during biofitration testing.

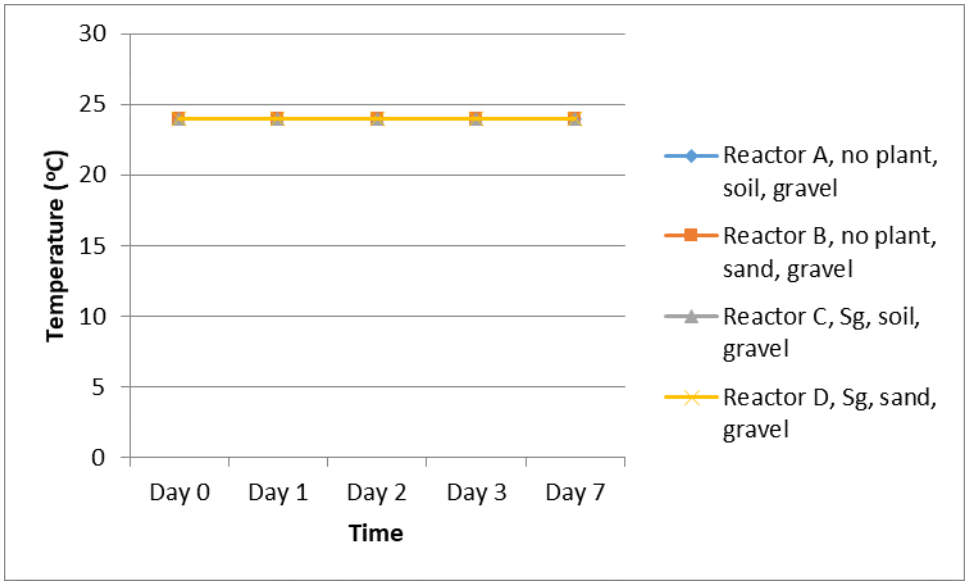

Figure 3 Temperature of every type of reactors during biofitration testing.

Removal of TSS, COD and TN

TSS results of every type of reactors during biofitration testing were presented in Figure 4. Based on that figure, TSS concentrations decreased during biofitration testing. TSS in reactor D -sand, gravel and plant of *S. grossus,* showed the smallest concentration of TSS at day 7. Although the differences of TSS concentrantion for 4 types of biofiltration reactor was not significant, it indicating that root of *S. grossus* and sand medium have a role in removal of TSS.

Figure 5 describe concentration of COD during biofitration testing for all biofiltration reactor. In general, concentration of COD desreased, although COD could increase at some days. Biofiltration system work as constructed wetlands. A grey water biofiltration system is a constructed wetland that removes a significant amount of pollutants from grey water before it flows into the ground water, river, or natural wetland (Gorky, 2015). According to Garcia et al. (2010), constructed wetlands are designed to remove the externally loaded

organic matter, but the internal loading must also be taken into account because it produces a background concentration that determines the maximum efficiency of the systems. The background concentration is usually represented as carbon. In terms of BOD or CODis often assumed to be around 3 mg/L (Rousseau et al., 2004). Plants seem to be the largest internal source of organic matter, and below-ground production is thought to generate more organic matter than above-ground production because the roots, rhizomes, leachates and exudates release matter directly into the granular medium (Tanner et al., 1998). As plants grow, die, and decay, dissolved organic matter can leach into the water. Bacterial decomposition of plant detritus converts particulate organic matter into humic substances that increase the bulk dissolved organic matter pool (Pinney et al., 2000; Quanrud et al., 2001).

Based on Figure 6, total nitrogen was reduced from 2.98 mg/L in initial grey water to 0.56 mg/L in reactor B and D. Both bacterial and plant utilization of nitrate nitrogen in the biofiltration reactor and the break down of organic nitrogen and subsequent release of ammonia to the air may account for the loss of N through the system (Travis et al., 2010).

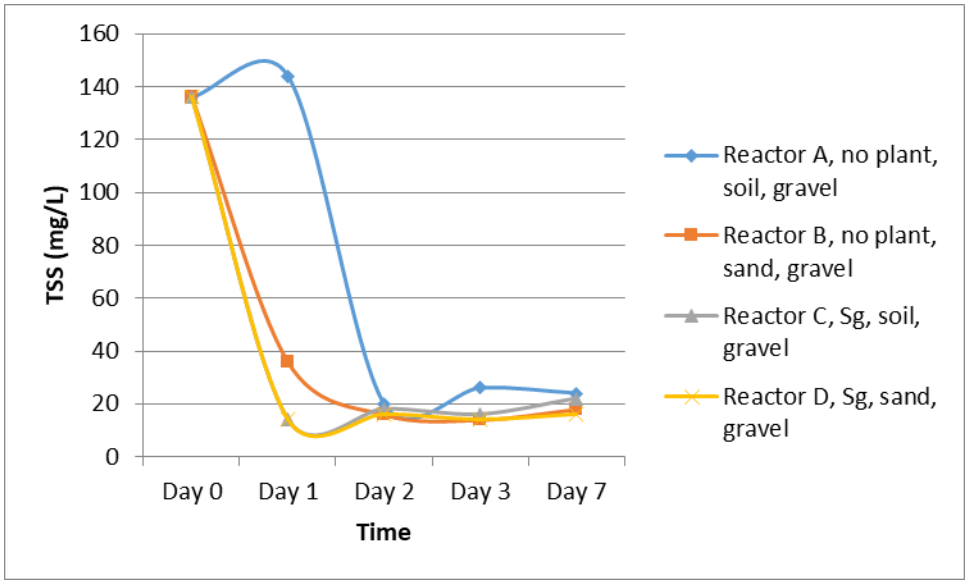

Figure 4. TSS concentration of every type of reactors during biofitration testing.

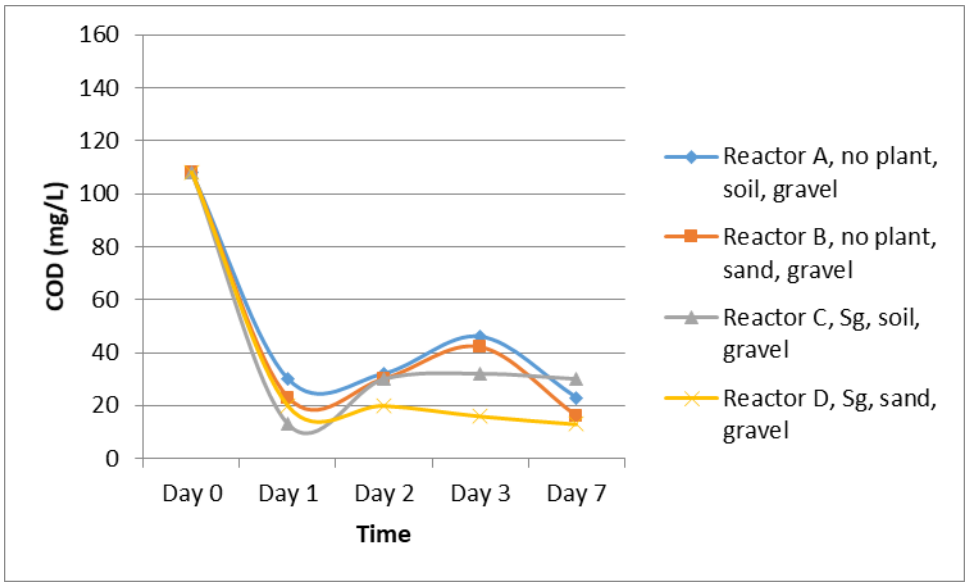

Figure 5 Temperature of every type of reactors during biofitration testing.

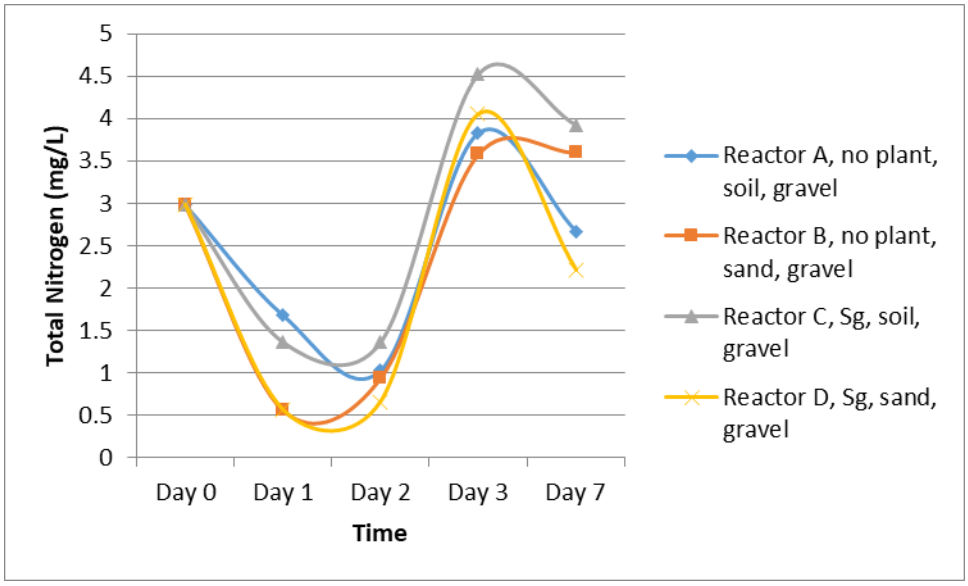

Figure 6 Temperature of every type of reactors during biofitration testing.

The performance of the biofilter system is evaluated in terms of comparison with *Peraturan Gubernur Jatim* No.72 in 2013. Based on *Peraturan Gubernur Jatim* No.72 in 2013, TSS concentration must below 50 mg/L and COD must below 50 mg/L. The concentration of TSS, COD and TN after day 7 in biofiltration reactor shown in Table 3. It showed that concentration of TSS, COD and TN were below the effluent standard. The biofilter recorded encouraging results in terms of percentages of removal. The results of the removal efficiency for parameters TSS, COD, and TN were illustrated in Figure7. The highest percentage removal of TSS, COD, and TN occured in reactor D, sand, gravel, and *S. grossus*, with removal were 88.2%, 88% and 77.9%, respectively. It indicating that *S. grossus*has a role to remove TSS, COD, and TN from grey water in biofiltration system.

Table 3. Concentration of TSS, COD and TN compared with effuent standard.

REACTORS	PARA-METERS	UNIT	DAY 0	DAY 7	PERGUB JATIM No. 72 IN 2013	REMOVAL (%)
Reactor A, no plant, soil, gravel	TSS	mg/L	136	24	50	82.4
	COD	mg/L	108	23	50	78.7
	TN*	mg/L	2.98	1.03		
Reactor B, no plant, sand, gravel	TSS	mg/L	136	18	50	86.8
	COD	mg/L	108	16	50	85.2
	TN*	mg/L	2.98	0.94		
Reactor C, Sg, soil, gravel	TSS	mg/L	136	22	50	83.8
	COD	mg/L	108	30	50	72.2
	TN*	mg/L	2.98	1.36		
Reactor D, Sg, sand, gravel	TSS	mg/L	136	16	50	88.2
	COD	mg/L	108	13	50	88.0
	TN*	mg/L	2.98	0.66		

*at Day 2

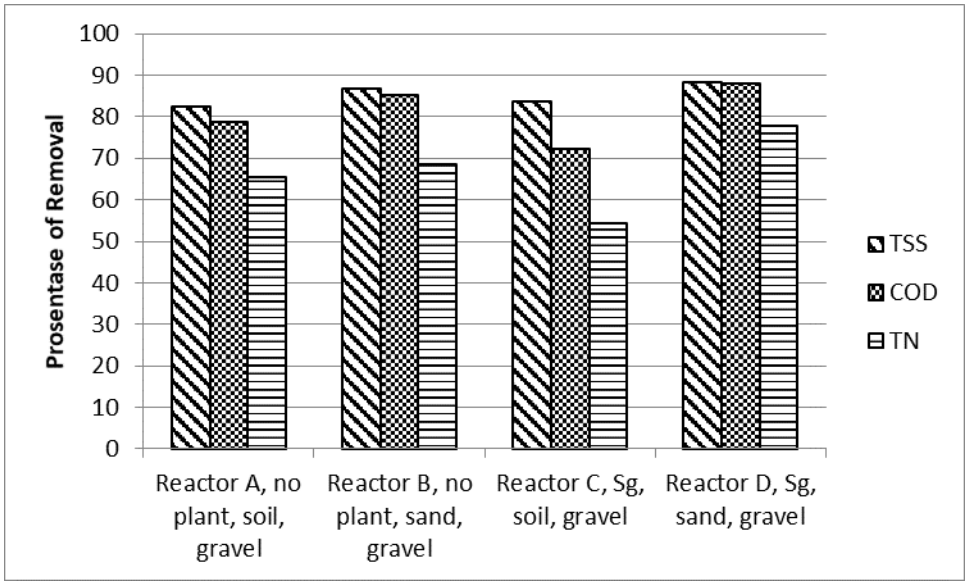

Figure 7. Percentages of TSS, COD and TN removal in every typeds of biofiltration reactor.

CONCLUSION

The highest percentage removal of TSS, COD, and TN occured in reactor D -sand, gravel, and *S. grossus*-, with removal were 88.2%, 88% and 77.9%, respectively. In, conclusion, plantof *Scirpus grossus* plays a role to remove TSS, COD,and Total Nitrogen from grey water in biofiltration system.

ACKNOWLEDGMENTS

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Beef Tenderization Using Bacterial Collagenase Isolated from Slaughterhouse

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ABSTRACT

Beef production in Indonesia is still below the market demand because of the low meat quality. The parameter that could increase the taste and sale price of the beef consist of tenderness, juiciness, color, and fat content. Tenderness can be influenced by many factors, such as the structure of muscle and connective tissue constituent, which can be degraded by using bacterial collagenase. Slaughterhouse waste is potentially used as the source of collagenase-producing bacteria. Thus, the study will be isolation and selection of bacteria from the waste that has the potential to produce collagenase for beef tenderization process. The extracellular protease with the novel property of hydrolyzing beef tendon was purified. The molecular mass of the purified collagenase was estimated as 72kDa. The optimum temperature and pH for the collagenase activity were 20 °C and pH=7.5, respectively. Soluble tendon was used as the substrate for determination of the soluble protein content. The obtained enzyme activity was 0,194 mg/ml. The thrust value difference was 21%. The study concludes that collagenase from isolated strain could be applied on the meat tenderization.

Keywords: **B**eef, collagenase, tenderness.

INTRODUCTION

Tenderness of beef can be influenced by many factors, such as the structure of muscle and connective tissue constituent (Calkins et al., 2007). One of the most abundant proteins in the beef muscle is collagen which has 65-85% proportion of the beef composition (Curwin, 1997). It has been reported that the beef collagenase can be degraded by using bacterial collagenase (Polkinghorne et al., 2008; Liu et al., 2010). The true collagenases are strictly defined as proteases capable of cleaving helical regions of collagen molecules in fibrillar form under physiological conditions of pH and temperature (Harrington, 1996). Many collagenolytic proteases have been reported originating from various microorganisms, such as *Clostridium histolyticum*, *Clostridium perfringens,Eubacteriumalactolyticum*, *Fusobacterium nucleatum*, *andVibrio vulnificus* (Fujimoto, 1975). Collagen as the main substrate of collagenase iscontained in animal connective tissue, such as skin, tendon, blood vessels, and bone (Robertson et al., (1972). Slaughterhouse waste is potentially used as the source of collagenase-producing bacteria. In this study, from the culture which isolated from animal tendon wastes, a novel collagenolytic protease has been purified. The results indicate the collagenasecan be used to degrade beef collagen and its potential to be applied on meat tenderization.

METHODS

Isolation and Selection of the Collagenolytic Bacteria

Microorganisms were isolated from the animal tendon wastes collected at Pegirian Slaughterhouse, Surabaya, Indonesia. These strains were screened on agar plates containing 6 g/L of tendon collagen, 7.5 g/L of agar, pH=7.0–7.5. The plates were incubated at 37 °C for 48 h. Colonies that grew well under such conditions were isolated and retained for subsequent screening. Eight bacteria with larger clear zone were obtained and then inoculated in the fermentation medium (in g/L): Tendon 40, Na_2PO_3 5, NaCl10, KH_2PO_4 1, Glucose 5, $MgSO_4.7H_2O$ 1, pH=7.0–7.2. The inoculum, 50 g/L, was added into baffled shake flasks (100 mL of liquid medium in 250-mL flasks). The flasks were shaken at 180 rpm on an orbital shaker at 21 °C. After 48 h of incubation, the culture broth was centrifuged (at 4 °C and 12000g for 5 min) and the supernatants were collected for enzyme activity measurement.

Growth Condition and Collagenase Production

Growth condition and enzyme production were used 500mL liquid medium containing (in g/L): Tendon 40, Na_2PO_3 5, NaCl10, KH_2PO_4 1, Glucose 5, $MgSO_4.7H_2O$ 1, pH= 7.0–7.2 was poured into the 2-litre baffled flasks for cultivation. The screened strain was inoculated (inoculum concentration of 40 g/L) and cultured at 21 °C for 72 h on a rotary shaker at 180 rpm. Samples were collected at 4-hour intervals in order to determine bacterial growth and collagenase activity. The growth was monitored by measuring A600 nm values.

Purification, Total Protein Content and Enzyme Activity Assay

All purification steps were carried out at 4 °C. After cultivation, the cells were removed by centrifugation at 12000 g for 10 min. Solid $(NH_4)_2SO_4$ was slowly added into the culture medium supernatant I (20mL) until the concentration reached saturation of 30 %. The volume of the resultant mixture increased,dissolved in Tris-HCl (pH=7.5) and was kept at 4 °C overnight. The supernatant II was collected by centrifugation at 12 000´g for 5 min. Solid $(NH_4)_2SO_4$was continuously added into the supernatant (30 %) until the concentration reached saturation of 45 %, stirred for 10 min, dissolved in Tris-HCl (pH=7.5) and left overnight at 4 °C. The supernatant III was collected by centrifugation at 12 000´g for 5 min. Solid $(NH_4)_2SO_4$ was continuously added into the supernatant (45 %) until the concentration reached saturation of 60 %, stirred for 10 min, dissolved in Tris-HCl (pH=7.5) and left overnight at 4 °C. The supernatant IV was collected by centrifugation at 12 000´g for 5 min. Solid $(NH_4)_2SO_4$ was continuously added into the supernatant (60 %) until the concentration reached saturation of 75 %, stirred for 10 min, dissolved in Tris-HCl (pH=7.5) and left overnight at 4 °C. The supernatant V was collected by centrifugation at 12 000´g for 5 min. Solid $(NH_4)_2SO_4$ was continuously added into the supernatant (75 %) until the concentration reached saturation of 90 %, stirred for 10 min, dissolved in Tris-HCl (pH=7.5) and left overnight at 4 °C.

Total protein content was estimated by using Bradford method (Bradford, 1976). Enzyme activity assay estimated by modifiedBradford method with BSA (*Bovine Serum Albumin*) as a standard that serves to illustrate the relation between concentration of the optical density. Initially with Bradford reagent manufacture of 100 mg *Coomasie Brilliant Blue* G - 250 was dissolved in 50 mL of 95% ethanol and added 100 mL of phosphoric acid 85 % (w/v). Then added distilled water to a volume of 1 liter and filtered with Whatman filter paper. Measurement of collagenase activity by adding 0.1 ml enzyme and 5 ml substrate into a test tube containing 5 mL of Bradford reagent. The absorbance was measured at 595 nm. The calculation of protein concentration by BSA standard curve concentration from 0 to 0.1 mg / mL with Bradford reagent.

Isoelectric Point and Molecular Mass Determination

Isoelectric point was measured using the method described by Burgess and Thompson (Burgess and Thompson, 2002). SDS-PAGE was carried out using the method described by Laemmli (1970) with 12 % polyacrylamide resolving gels. Protein bands were visualized by staining with Coomassie Brilliant Blue R-250.

Beef Tenderness Assay

Two slices of meat with dimensions of 2 x 2 x 2 cm wasimmersed in the enzyme solutionand the other was immersed with water for 15 minutes at the room temperature. The strength of the sample of 20mm thick steak was measured when pierced to a depth of 10 mm by a penetration test jig of diameter 5 mm at a speed of 100 mm/min. The test was done by measuring the thrust of material using Shimadzu penetrometer with Newton (N) unit parameter.

RESULT AND DISCUSSION

Isolation and Selection of the Collagenolytic Bacteria

The selection, isolation, and purification of bacteria that have the potential of producing collagenase in the slaughterhouse waste produced 16isolates. The purification was conducted using the streakplate method to obtainthe pure colonies. Sixteen isolates were tested its collagenolytic ability using clear zone method. The test resulting 8 positive isolates (KLG-1, KLG-2, KLG-3, KLG-4, KLG-5, KLG-6, KLG-7, and KLG-8)

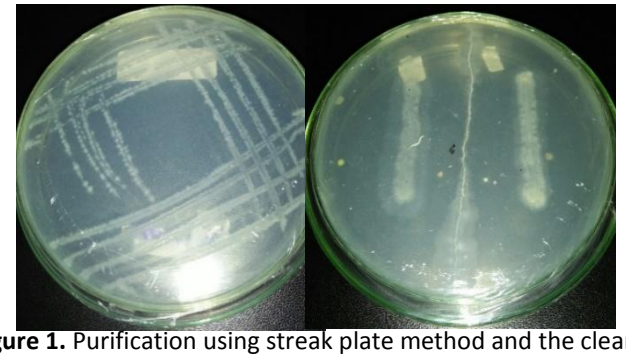

Figure 1. Purification using streak plate method and the clear zone test.

The clear zone resulted in collagen medium was the representation of the collagenase activity (Doust and Mobarez, 2004). The ratio of the clear zone on each isolate can be observed in Figure 2. Figure 2 shows that KLG-8 has the highest clear zone ratio, while the isolate with the lowest ratio was KLG-2. Nonetheless, the results of this test can't be indicated as the collagenase activity. The enzyme activity will be discussed furtherin activity section.

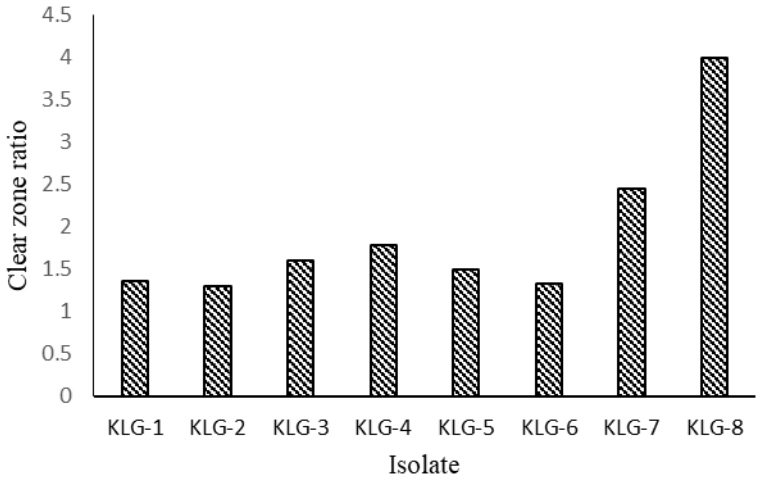

Figure 2. Clear zone ratio.

The clear zone was the visualization of enzymatic activity around the colonies after the strain inoculated in collagen agar (Wu et al., 2010). The ratio of clear zone was determined by dividing the total area of activity (the area of the clearing zone less the area of the colony) with the area of the colonies (Wu et al., 2010). Collagenolytic isolate morphology was observed with simple staining method using methylene blue to differentiate the cells. The result shows that each isolate was pure.

Growth Condition and Collagenase Production

A time course study was performed in order to determine the bacterial growth and collagenase production. In Fig. 2, bacterial growth (expressed as ln A=600 nm) determined at 2-hour intervals, are presented. The maximal growth of this bacterium was achieved after 64h of incubation (Figure 3). The isolates relative grew exponentially from 12–44h. After 60h of culturing, the specific growth rate of bacteria decreased. After 44 h, the growth is no longer exponential because the most preferred medium components have been depleted and bacteria started to secrete collagenase (Liu et al., 2010).

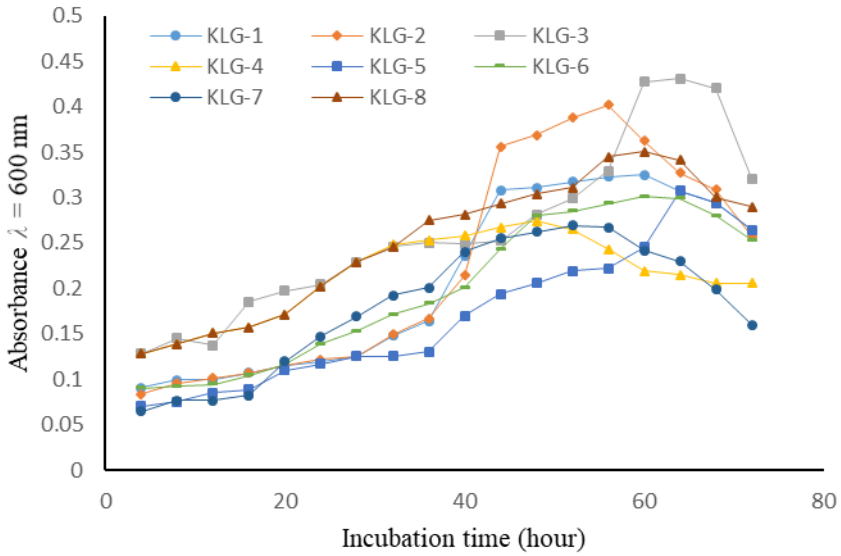

Figure 3. Growth profile of collagenolytic bacteria.

The results indicate that not every isolate has normal growth phase. The best growth was shown by KLG-6 and KLG-8. The growth of collagenolytic bacteria has exponential growth period up to 72 hours before entering the death of up to 144 hours (Waldvogel and Swartz, 1969). The enzymes were harvested between the late logarithmic phase and the early stationary phase of each isolate. Production of the enzyme normally occurs in the late logarithmic phase, when the cell density is high (Zaliha et al., 2005). The result (table 1) shows that KLG-7 has the highest enzyme activity with 0.194 mg/ml/sec. KLG–4 has low enzyme activity despite the high soluble protein content. KLG-5 soluble protein content value was lower than the spectrophotometer detection limit and considered not measurable.

The difference result between enzyme activity assay and the clear zone test showed that the eight isolates had different tendencies of enzyme production. The high result of clear zone test does not affect the enzyme activity, vice versa. Allegedly, it caused by the bacteria's ability to obtain nutrients in the agar and liquid medium.

Table 1. Collagenase activity and soluble protein content.

Isolate	Enzyme Activity (mg/ml/sec)	Protein Content (mg/ml)
KLG-1	0.155	0.021
KLG-2	0.136	0.021
KLG-3	0.175	0.023
KLG-4	0.136	0.049
KLG-5	0.155	unmeasurable
KLG-6	0.175	0.018
KLG-7	0.194	0.019
KLG-8	0.175	0.011

Purification, Total Protein Content, and Enzyme Activity Assay

In the presence of tendon collagen as the major carbon and nitrogen source, the isolatessecreted collagenase into the culture medium. The secreted collagenase was purified with five fractions of purification steps using ammonium sulfate (Figure 4).

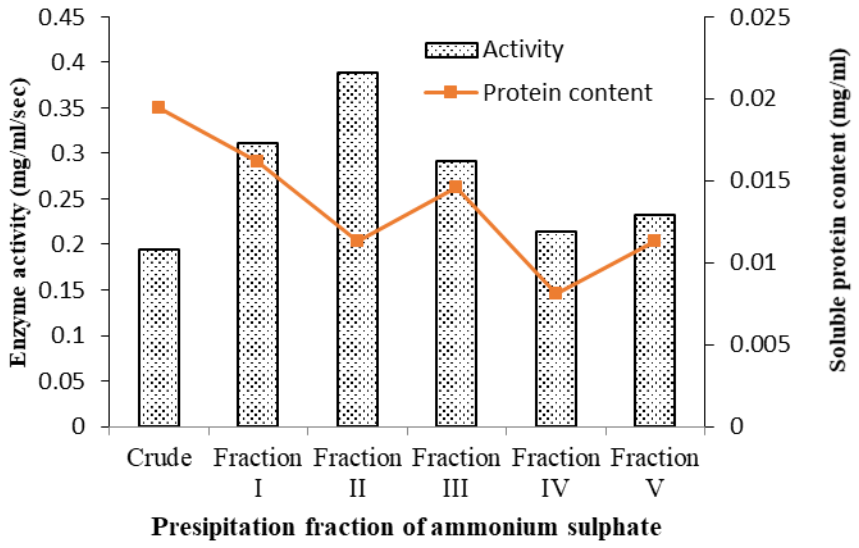

Figure 4. Enzyme activity and soluble protein content before and after purification.

In Figure 4, it can be observed that the fraction II has the highest activity of collagenase (0.388 mg/ml/sec), this result shows that the fraction has the high accumulation of collagenase protein. While on the other fraction is relatively lower protein activity showed that the majority of the precipitated protein is not collagenase. Ammonium sulfate precipitation method aims to attract the water molecules from the pockets of the hydrophilic molecule in the protein so that the similar proteins will bond with Van Der Waals forces and cause precipitation (Miller, 2007).

Isoelectric Point and Molecular Mass Determination

Characterization of collagenase in the highest yield (KLG-7) precipitation was done by varying the pH to determine the isoelectric point (pI).The isoelectric point (pI) was achieved at pH=6 (Figure 5). Collagenase has the isoelectric point value in the pH range of 5.35 to 6.20 (Bond and Van Wart, 1984). Isoelectric point isa condition when protein molecule has the same number of positive and negative charges, which resulted in coagulation at the specific pH level (Pelegrine et al., 2004).

Figure 5. Coagulated protein of collagenase at pH 6.

The molecular mass determination was tested by using SDS-PAGE (Figure 6) to determine the level of molecular weight protein which can be used as enzymes confirmation resulted from partial purification. In Figure 6, lane 1 wasthe marker of protein with specific molecular weight, while lane 2 was KLG-7 fraction II. The determination of the molecular weight of collagenase was carried by dividing the migration distance of the protein sample with maximum distance migration of protein marker multiplied by the maximum mass of the marker.

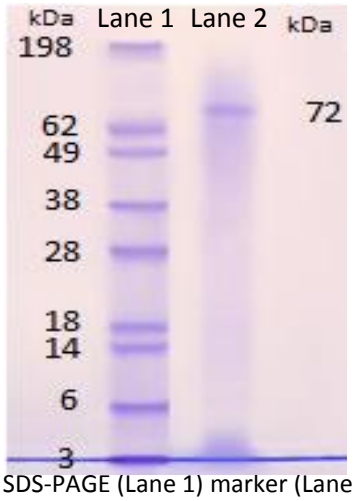

Figure 6. SDS-PAGE (Lane 1) marker (Lane 2) KLG-7 fraction II.

The molecular mass of the purified collagenolytic enzyme was 72 kDa. This result was smaller than the molecular mass of the collagenases isolated from C. histolyticum, C. perfringens, and Acinetobacter sp. (molecular masses of 120, 66-125, and 102 kDa) [4]. Also, the molecular mass of the protease isolated in the present paper was lower than the molecular mass of collagenolytic proteases purified from *Bacillus cereus* by Mäkinen et al. (1987) and Lund et al. (1999), which had molecular masses of 87 and 105 kDa, respectively. The molecular mass of this preparation was close to the molecular mass of proteases isolated from Vibrio alginolyticus (82 kDa) (Hare et al.,J. 1983).

Beef Tenderness

The level of meat tenderness was represented as force unit of the material thrust which measured by Shimadzu penetrometer with Newton parameter (Figure 7). Two sample of brisket meats were cut cuboid with dimensions of 2 cm x 2 cm x 2.The result will be generated into penetrogram and thrust value. The greater the thrust value shows that the meat has the harder texture, vice versa.

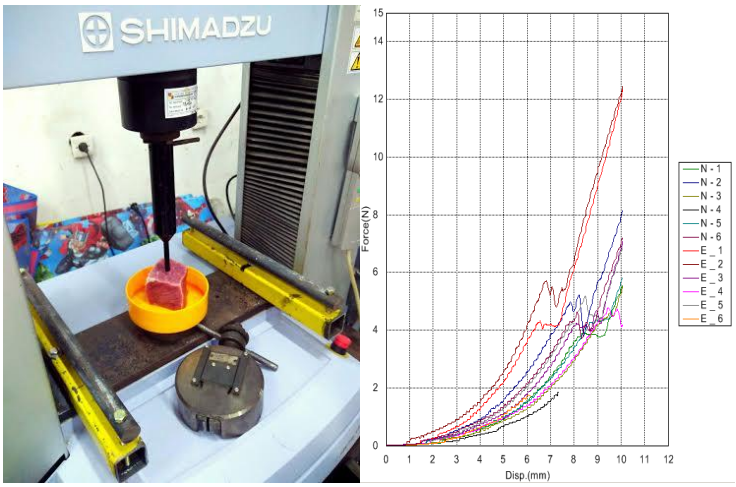

Figure 7. Tenderness test penetrometer and penetrogram.

In figure 7,the sample which immersed for 15 Minutes in enzyme solution has thrust value of 6.24 N while the other sample which immersed in wateronly has thrust value of 9.66 N. The test results show that the samples of the meat with the enzyme have 21% lower thrust value. The lower thrust value of the sample was caused by the structure of the meat becomes tenderer through degradation process on collagen layer by collagenase. Resulted in themodification of meat structure by losingthe connective tissue components. Modification of connective tissue is desirable to reduce toughness of meat since the quantity and degree of cross-linkage of connective tissue affects the meat tenderness (Goll et al., 1964).

CONCLUSION

Eight bacteria which isolated from slaughterhouse waste were able to produce collagenase. The highest collagenase activity achieved by KLG-7 with the activity of 0.388 mg/ml/sec for each 1 ml partially purified enzymes. Produced collagenase hasthe soluble protein concentration of 0.011 mg/mL with the isoelectric point at pH 6. The molecular weight results from SDS-PAGE analysis was 72 kDa. Collagenase is able to tenderize meat produced with 21 % 21% lower thrust value and has the potential to be applied on meat tenderization.

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Spectrophotometry and Turbidimetry Approximation Approach On *Nannochloropsis* Sp. and *Chlorella* Sp. Biomass In Cultivation Stages

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ABSTRACT

Cell density quantification, both in axenic or xenic culture of microalgae, determines it biomass. Rapid quantification method of microalgae biomass is important to explore. The aim of this research was to asses *Nannochloropsis* sp. and *Chlorella* sp. biomass using spectrophotometry and turbidimetry methods compared with cell density quantification. *Nannochloropsis* sp. and *Chlorella* sp. was cultured in laboratory scale during six days. Absorbance, turbidity, and cell density were analyzed every day. Absorbances were analyzed on 630, 647, 664, 665, and 750 wavelengths. The result showed that absorbance and turbidity increased along with the increasing of cell density in both culture of microalgae. The absorbance in fifth wavelength showed similar pattern. Cell density of *Nannochloropsis* was ranged from $1.91x10^6$ to $4.20x10^6$ cell/m^3 and *Chlorella* was ranged from $1.09x10^6$ to $2.84x10^6$ cell/m^3. The range of turbidity was 17.2-57.4 NTU on *Nannochloropsis* and 9.69-54.1 NTU on *Chlorella*. The absorbance and turbidity value showed high correlation with cell density. Spectrophotometry and turbidimetry methods are a good approximation approach for microalgae biomass quantification.

Keywords : Absorbance, Cell density, *Chlorella, Nannochloropsis*, Turbidity

INTRODUCTION

Plankton is a very small organism and free floating live in the water. Plankton consists of two major categories, phytoplankton and zooplankton (Sumich, 1999). Phytoplankton (microalgae) is a major producer (primary producer) of organic substances in aquatic ecosystems that has a very important role in aquatic ecosystems. Microalgae such as *Chlorella* sp. could be utilized as fish natural feed because of containing 50 % protein, fat, and vitamin A, B, D, E, and K Kawaroe (2010). Similar to *Chlorella* sp., *Nannochloropsis* sp. also could be used as food source for fish larvae and rotifers because it contains rich energy.

Nowadays, culture of microalgae has been rapid developed because of industrial demand. In microalgal culture, control of cell density is an important factor. Normally, cell density of microalgae is quantified by direct cell observation under microscope. This method can give accurate result, yet this method needs high precision and longer observation time. However, it is required faster, easier, and precise quantification methods to make more efficient culture activity.

Several researches have already quantified microalgal cell abundance using spectrophotometer device (Kiefer *et al*. 1979; Sorokin, 1979). In measurements of exponential rate as increase in optical density (O.D.) percent transmission for a growing cell suspension is determined at intervals with a measuring device (spectrophotometer, colorimeter, or similar instrument suitable for measurements of the increase in turbidity of microbial suspensions) (Sorokin, 1979). Turbidity in water is caused by suspended and colloidal matter such as clay, silt, finely divided organic and inorganic matter, and plankton and other microscopic organisms (APHA,

2012). Turbidity is an expression of the optical property that causes light to scattered and absorbed rather than transmitted with no change in direction or flux level trough the sample.

Information of spectrophotometer and turbidimeter application in assessing microalgal cell abundance is still limited. Hence, this research was aimed to asses *Nannochloropsis* sp. and *Chlorella* sp. biomass using spectrophotometry and turbidimetry methods compared with conventional cell density quantification.

LITERATURE REVIEW

Microalgae

Nannochloropsis is a genus of algae that have mostly been known from the marine environment but also occur in fresh and brackish water (Fawley and Fawley, 2007). All of the species are small, non motile spheres which do not express any distinct morphological feature, and cannot be distinguished by either light or electron microscopy. *Nannochloropsis* differ from other related microalgae in that they have chlorophyll-a and completely lack chlorophyll-b and c. In addition they are able to build up a high concentration of a range of pigments such as astaxanthin, zeaxanthin, and canthaxanthin (Lubian, 2000).

Nannochloropsis is considered a promising alga for industrial applications because of its ability to accumulate high levels of polyunsaturated fatty acids (Sukenik *et al*., 1989). Moreover, it shows promising features that can allow genetic manipulation aimed at the genetic improvement of the current oleaginous strains. Various species of *Nannochloropsis* indeed are transfectable and there has been evidence that some strains are able to perform homologous recombination (Kilian, 2011).

Chlorella sp. is a single-celled alga (unicellular), microscopic, cell diameter of 2-8 micrometer sized, ball-like round and oval (Presscott, 1970). *Chlorella* sp. is green colored because the cells containing chlorophyll in large numbers, in addition carotene and xantofil. There are core and coated membranes of chloroplasts. The chloroplasts have the stigma that is sensitive to light. *Chlorella* sp. including the type of algae that can perform photosynthesis because it has some pigment chlorophyll-a, chlorophyll-b, chlorophyll-c, carotene and xantofil (Borowitzka, 1988).

The density of microalgae is the amount of microalgae in the void volume (cell/ml). Density of phytoplankton in the waters is affected by several environmental parameters and physiological characteristics. The composition and abundance of phytoplankton will change at various levels in response to changes in environmental conditions such as physical, chemical, and biological (Reynolds, 1984).

Of all indices of growth, measurements of optical density (turbidity technique) are particularly suitable for determination of growth rate. The basic advantage of the turbidity technique in growth rate measurement, in addition to its efficiency, is the possibility of taking repeated readings on the increase in turbidity of the same batch of the suspension of microbial cells. The possibility of avoiding sampling by the turbidity technique saves time and increase the accuracy of growth measurements (Sorokin, 1979).

In measurements of exponential rate as increase in optical density (O.D.) percent transmission for a growing cell suspension is determined at intervals with a measuring device (spectrophotometer, colorimeter, or similar instrument suitable for measurements of the increase in turbidity of microbial suspensions) (Sorokin, 1979).

Spectrophotometer

Spectrophotometer as the name suggests is a tool that consists of spectrometers and photometer. Spectrophotometer produce beams of a specific wavelength spectrum and the photometer is measuring device transmitted light intensity or absorbed. Spectrophotometer is used to measure the relative energy if the energy is transmitted, reflected, or emitted as a function of wavelength (APHA, 2012).

The electromagnetic spectrum is divided into several areas of light. An area will be absorbed by the atom or molecule and the wavelength of light absorbed can show the structure of the compounds studied (APHA, 2012). Absorption spectrum in the areas of ultra violet and visible light generally consists of one or several wide absorption band; all molecules can absorb radiation in the UV-visible region. Therefore they contain electrons, either shared or not, which can be excited to a higher level. Wavelength at the time of absorption occurs depends on how tightly bound electrons in the molecule (Wunas *et al*., 2011). The main advantage spectrophotometric method is that this method provides a simple way to determine the quantity of a substance which is very small (Nur *et al*., 2012).

Turbidimeter

Turbidity is an optical measurement of scattering light produced. Ray scattering occurs due to interactions between the light given by the suspension of particles dispersed in solution. The particles can be the suspension of algae clay, organic material, microorganisms, colloidal material and even though large molecules such as tannins and lignins (APHA, 2012). The general principle of the tool is a ray coming turbidimeter a particle there is continued and there is reflected, the light that passed was used as the measurement basis (Day and Underwood, 2002).

MATERIALS AND METHODS

Microalgae cultivation

Microalgae were cultured in Plankton Research Laboratory, Department of Aquatic Resources Management, Faculty of Fisheries and Marine Science, Bogor Agricultural University, West Java, Indonesia. The two inoculums of microalgae, *Nannochloropsis* sp. and *Chlorella* sp were used for experiment.

Nannochloropsis sp. was cultivated in batch cultures by inoculating 50 ml of sterile artificial medium with 50 ml axenic strain. Seawater Provasoli medium was used as artificial medium for *Nannochloropsis* culture. Microalgae were placed in sterile flasks (200 ml), one flask for one day experiment.

Cultivation of *Chlorella* sp. was conducted using the same technique as *Nannochloropsis*, except for artificial medium. The *Chlorella* medium was freshwater Provasoli. These microalgae were grown in continuous culture under 2110-2500 Lux light intensity and 25°C controlled temperature.

Cultivation was performed during 6 days. Daily observation of microalgae cell density was conducted using cell counting method and optical density using spectrophotometry and turbidimetry methods.

Cell density quantification

Cell of microalgae from each flask was taken by sterile disposal pipettes. Cell density of *Nannochloropsis* was counted using Hemocytometer with 0.1 mm deep. Cell density formula was described by Guillard (1979) as follows.

$$d = 5 \times 10^3 \times P$$

with:

P = average number of organisms per square

Morphology of cells was observed every day and documented with Axio Cam ERc5s t. The growth rate (Sorokin 1979) and division time (Guillard 1979) is calculated using these following equations.

$$R_E = \frac{\ln X_2 - \ln X_1}{t_2 - t_1}$$

with:

R_E = exponential growth rate

X_1, X_2 = the numerical values for cell mass at the begin and end of the period which exponential growth rate is constant

t_1, t_2 = corresponding times, at which X_1 and X_2 values are determined, in terms of the unit of time used in calculations

$$Dt = \frac{0.6931}{R_E}$$

with:

Dt = doubling time

Optical density analysis

Spectrophotometry analysis of *Nannochloropsis* sp. and *Chlorella* sp. culture was conducted using 630, 647, 664, 665, and 750 wavelengths. Spectrophotometer UV-1800 Shimadzu specification was used in this experiment. These wavelengths were related to chlorophyll analysis (APHA, 2012).

The samples of microalgae cell was taken by sterile pipette and placed in plastic clean cuvettes. The cuvettes were placed immediately in spectrophotometer. This method also described by Kiefer *et al*. (1979), cuvettes were placed either immediately in front of the integrating sphere so that all forward flowing light was collected (diffuse transmittance) or immediately behind the integrating sphere so that all backward flowing light was collected (diffuse reflectance).

Turbidity was analysed using HACH 2100Q and described by standard method APHA (2012). Samples of microalgae were taken by sterile pipette and placed in plastic clean cuvettes. Turbidity measurement must be undertaken immediately to prevent temperature changes and particle flocculation and sedimentation from changing sample characteristics (APHA, 2012).

RESULTS AND DISCUSSIONS

Results

Growth pattern of *Nannochloropsis* and *Chlorella* in six days observation had lag phase, exponential growth, and declining phase (Fig 1). Cell density was increased along with the increasing of the age of culture. In batch culture, which nutrient condition is limit, on 6th day cultivation, *Nannochloropsis* showed decreasing of cell abundance or start to the death phase. The specific growth rate, which means exponential growth rate (μ) of the *Nannochloropsis* cells, is 0.25. This value corresponds to doubling time value (2.79).

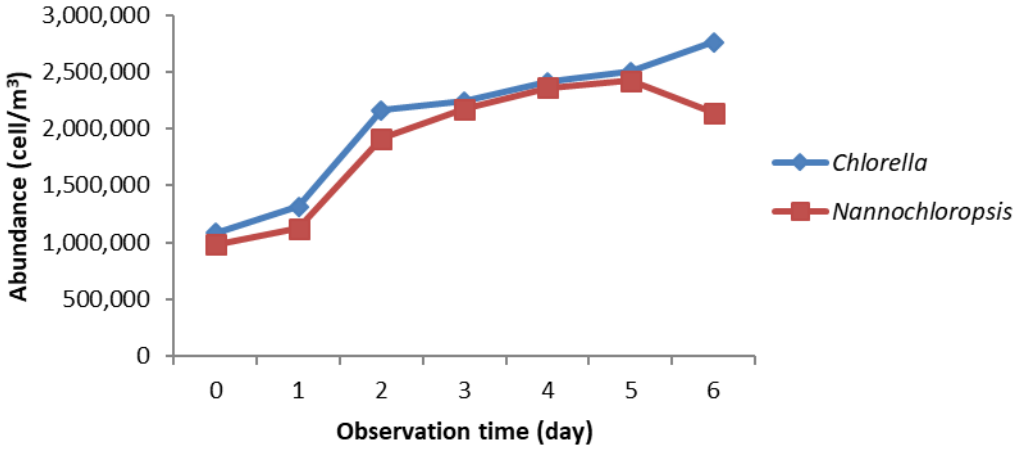


Figure 1. Growth phase of *Nannochloropsis* and *Chlorella* in laboratory culture.

Chlorella growth pattern in batch culture was similar to *Nannochloropsis*, except on 6th day, cell abundance increased. Even though cell abundance of *Chlorella* inoculum was higher than *Nannochloropsis*, the specific growth rate of *Nannochloropsis* was higher than *Chlorella* sp. μ of *Chlorella* was 0.20 and the doubling time was 3.43.

Approximation of cell age using optical density was showed in Fig. 2. Absorbance of *Nannochloropsis* samples were increased until fifth day experiment and decreased on sixth day. Similar to spectrophotometry result, turbidity from *Nannochloropsis* samples also increased until fifth day experiment. Correlation between absorbance in all wavelengths and age of culture (observation time) and between turbidity and age of culture was high, r > 0.90 and r > 0.90, respectively.

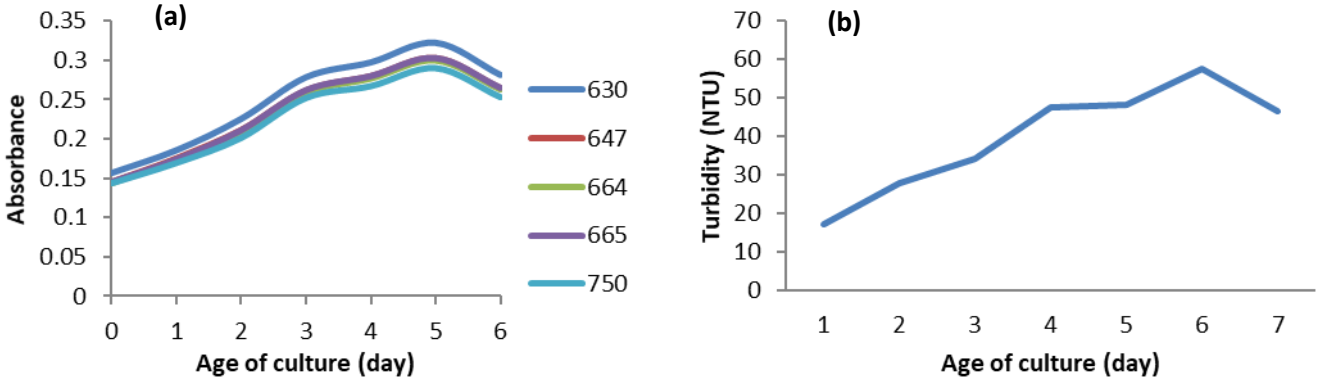


Figure 2. (a). Approximation of *Nannochloropsis* age culture based on optical density
(a) Absorbance of spectrophotometer, (b) Turbidity.

Estimation of cell abundance or density based on optical density measurement was described in Fig. 3. Application of linear regression analysis on absorbance versus cell abundance data showed high correlation in five wavelengths (r > 0.90). Determination coefficient (R^2), which means the models were represent 97% to culture condition. Turbidity of *Nannochloropsis* also showed high correlation (r >0.90) and determination coefficient was 99%.

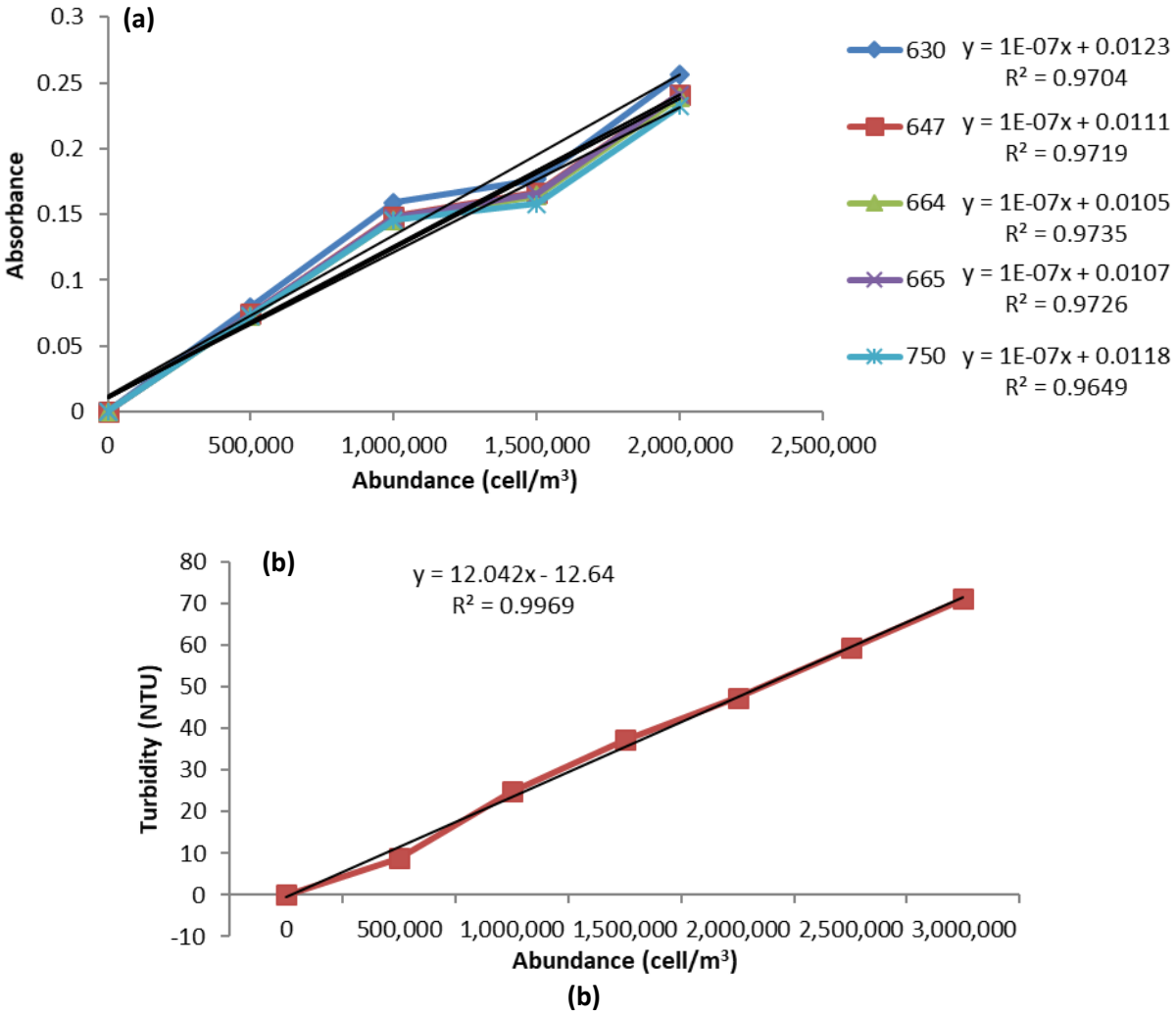


Figure 3. Linear regression for determining *Nannochloropsis* abundance using (a) Spectrophotometer, (b) Turbidimeter.

Approximation of *Chlorella* cell age using optical density was showed in Fig. 4. Different with *Nannochloropsis*, absorbance of *Chlorella* samples increased until the end of experiment. Turbidity from *Chlorella* samples also showed the same pattern, increased until sixth day experiment. Both absorbance and turbidity to age of culture (observation day) showed high correlation ($r > 0.90$).

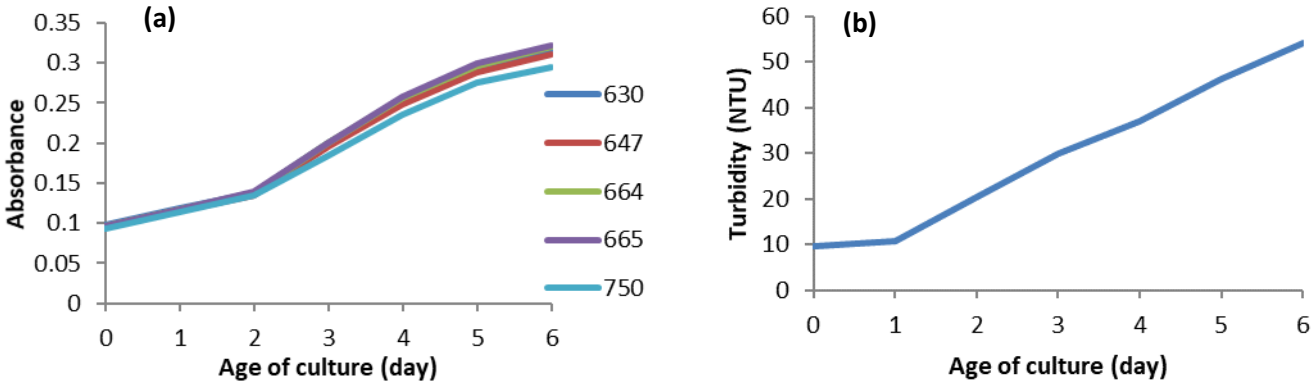


Figure 4. Approximation of *Chlorella* age culture based on optical density
(a) Absorbance of spectrophotometry, (b) Turbidity.

Estimation of *Chlorella* cell abundance or density based on optical density measurement was described in Fig. 5. Application of linear regression analysis on absorbance versus cell abundance data show high correlation between abundance and absorbance in five wave length ($r > 0.90$). Determination coefficient (R^2), which means the models were represent 99% to culture condition. Turbidity of *Chlorella* also showed high correlation ($r > 0.90$) and determination coefficient was 93%.

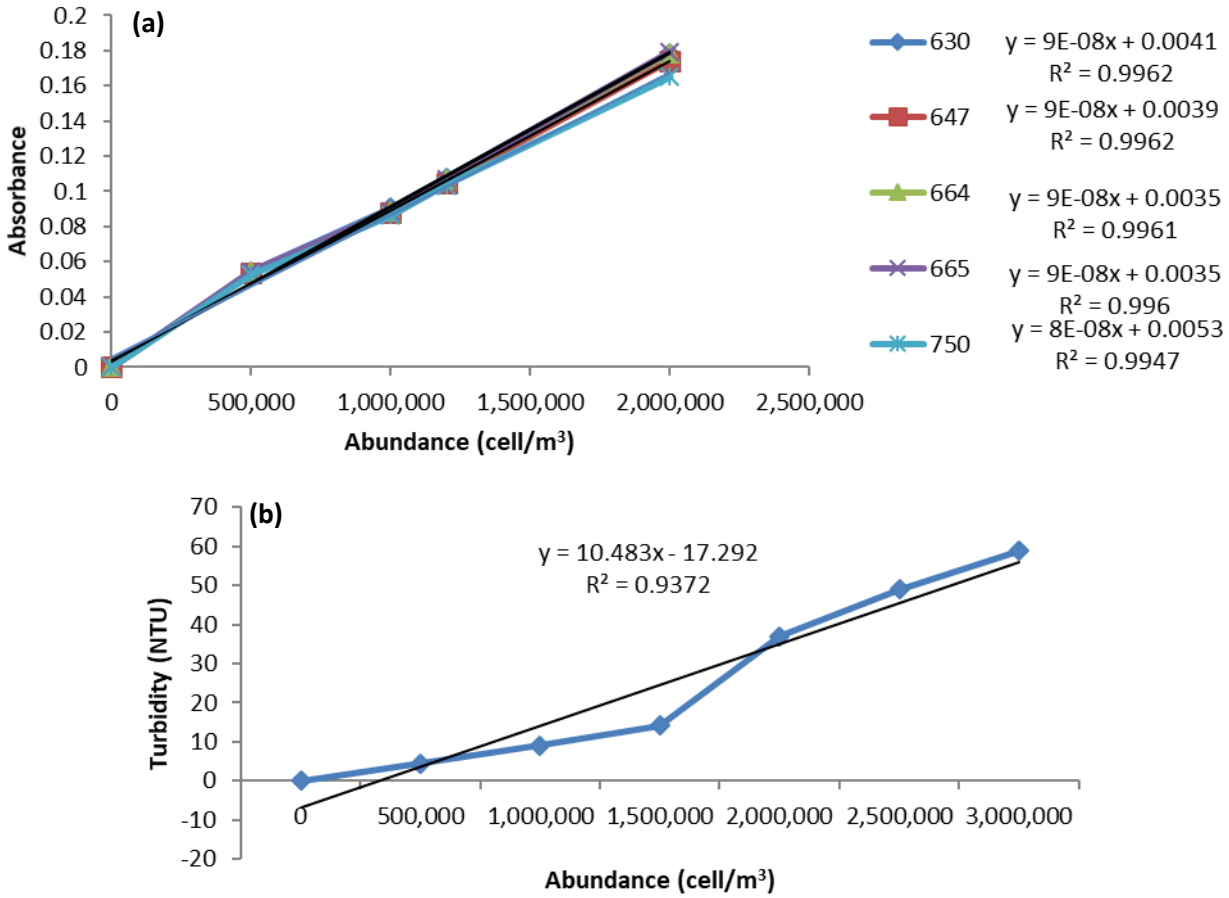
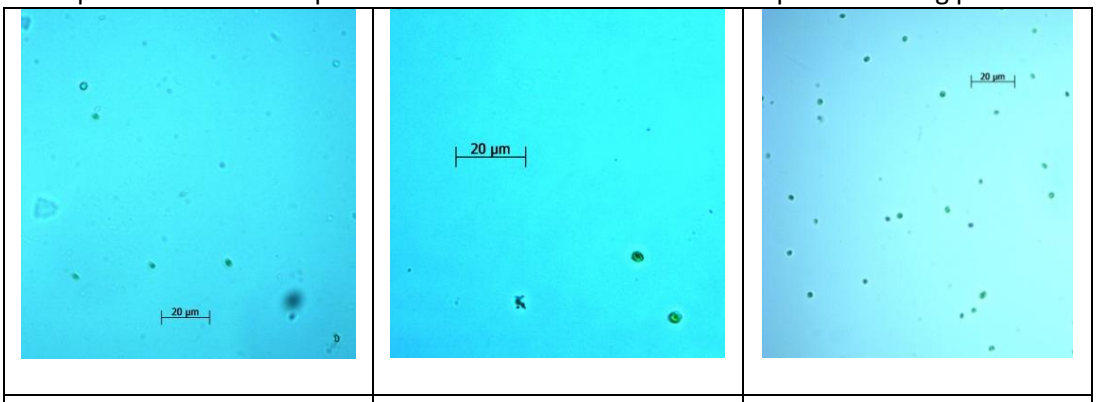


Figure 5. Linear regression for determining *Chlorella* abundance using
(a) Spectrophotometer, (b) Turbidimeter.

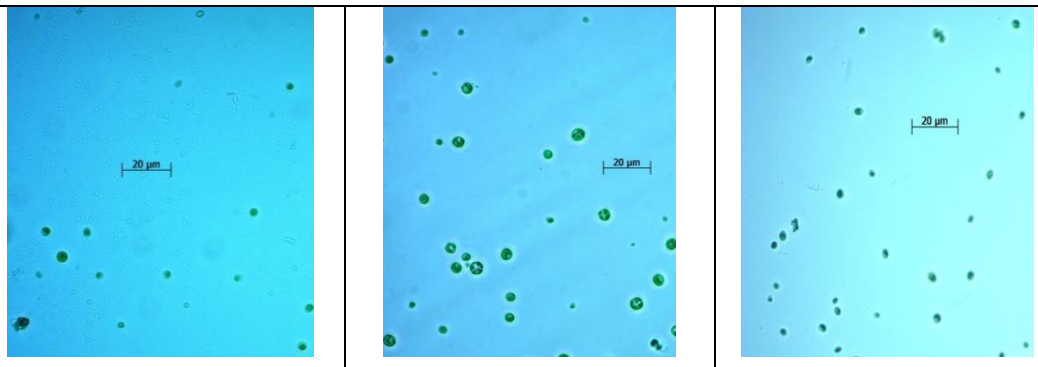
DISCUSSIONS

Growth rate phase of cells-culture of *Nannochloropsis* in seawater Provasoli medium in six day experiment showed lag phase in first day after inoculation, then exponential phase until fourth day, and then declining phase. Similar to *Nannochloropsis*, *Chlorella* exponential growth rate was reached from second day after inoculation until fourth day. Exponential growth rate was characterized by doubling time of cell (Menzel and Dunstan, 1979). Ability of *Nannochloropsis* cells doubled was shorter than *Chlorella* sp. The shorter doubling time indicates the higher productivity. The changes of growth phase determines by changes of morphology cells (Fig. 6.). In lag phase, cell size was small. In exponential growth, cell size became bigger and nucleus of cell was clearly in oval shape. Cells were clumped and nucleus was in shoe horse shape in declining phase.



Cell in lag phase-innoculation day (H0)	Cell in exponential phase-day 1-4 (H1-H4)	Cell in declining phase-day 5 (H5)

(a)



Cell in lag phase-innoculation day (H0)	Cell in exponential phase-day 1-4 (H1-H4)	Cell in declining phase-day 5 (H5)

(b)

Figure 6. Cell morphology characteristics of (a) *Nannochloropsis* and (b) *Chlorella* under microscope observation during 6 days.

During the lag phase, growth rate is changing, usually increasing with time. However, during portion of the lag phase, net growth may be absent or they may even be a decline in cell mass (dry weight of cells, turbidity of cell suspension, etc.) per unit volume of cell suspension (Guillard, 1979). The statement was proofed by the increasing of optical density, both absorbance of spectrophotometry and turbidity from the first day inoculation until the end of observation. In exponential phase, both absorbance and turbidity of *Nannochloropsis* and

Chlorella increased. Even though optical density increased after exponential phase, the growth rate was in declining phase. The clumped cell in declining phase or in steady state also result higher absorbance and or turbidity.

Estimation approach of absorbance analysis to cell abundance quantification was shown in Fig. 3 and 5, when cells abundance dilutes in certain volume, the absorbance will also decrease. Appropriateness of estimation also measured by r value, which mean spectrophotometry analysis could be applied for cell abundance estimation. The same as turbidity, when cells abundance diluted in certain volume, the turbidity decreased. This means turbidity could be used as cell abundance approximation.

CONCLUSION

The absorbance in 630, 647, 664, 665, and 750 nm and turbidity showed high correlation with cell density of *Nannochloropsis* and *Chlorella* ($r > 0.90$). Spectrophotometry and turbidimetry methods are a good approximation approach for microalgae biomass quantification.

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Production and Purification of Lipase with Intestinal Cattle Fat as A Substrate Using Submerged Fermentation

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ABSTRACT

The Intestine is one of solid waste in slaughterhouses. intestine had role to synthesis triacylglycerol (triglyceride; TAG). Intestinal TAG synthesis can contribute to excessive accumulation of TAG in tissues. The TAG or fat became a specific substrate for lipase production. Studies on lipase production were carried out with a bacterial strain (*Bacillus* sp. SKII-5) which isolated from Dieng Plateau Creater, Central Java, Indonesia. In this research was utilized intestine cattle fat as a substrate for lipase production through submerged fermentation. The substrate have variation of concentration is 0.11%, 0.21%, 0.31% and 0.42%. Lipase activity was measured using p-nitrophenyl palmitate (pNPP). Among the concentration, 0.11% fat in medium was found to be yielded maximum lipase and hence it was selected as a major substrate for further study. The highest lipase activity is 2858.6 U/ml. This enzyme was purified using ammonium sulphate precipitation conducted with 15-30%, 30-45%, 45-60%, 60-75% and 75-90%, Results revealed that 60-75% saturation was proved to be effective for maximum lipase activity of 1642.6 U/ml. Lipase produced in high temperature, it was include thermostable enzyme.

Keywords: *Bacillus* sp. SK II-5, intestinal cattle fat, tipase, thermostable.

INTRODUCTION

Lipase or *triacylglycerol acylhydrolases* (EC3.1.1.3) are enzyme that dissolve in water which catalyses the hydrolysis of fatty acid carboxyl ester bond in the triacylglycerol (TAG) and synthesis of long-chain acylglycerols with trioleoylglycerol being the standard substrate. The lipases catalyze wide range of reactions, including hydrolysis, inter-esterification, alcholysis, acidolysis, esterification and aminolysis (Jaegert and Eggert, 2002; Vakhlu, 2006). Lipases are unique in catalyzing the hydrolysis of fats into fatty acids and glycerol at the water–lipid interface and reversing the reaction in non-aqueous media (Saxena *et al.,* 2003). Lipases are widely used in the detergents indusrty, food processing, paper manufacture, the synthesis of fine chemicals and pharmaceuticals, bioconvertion and waste water treatment, and oleochemichal industry (Sharma *et al*., 2001). Microbial lipases are receiving a great deal of attention due to their potential for use in industrial processes (Houde *et al*., 2004) high yield and low production cost, diversity in catalytic activities, amenability to genetic manipulation, stability in organic solvents and broad substrate specificity. Microorganism which able to produced lipase was from the group of yeast *Candida rugosa, C. antarctica C.viswanathii*, groups of fungi *Aspergillus* sp., *Penicillium* sp., *Mucor*

sp., *Fusarium* sp., Actinomycetes and bacteria (Vakhlu *et al*., 2006; Gunasekaran and Das, 2005); Mohan *et al.,* 2008., Papanikolaou *et al*., 2011; de Almeida *et al*., 2013). Bacteria strain had greater potential to produced lipase because of the enzymes activity was higher than other microorganism (Bayoumi *et al*., 2012; Jaeger *et al*., 1999). Lipolytic bacteria is capable to produce lipase (Jaeger *et al*., 1999) one of that from genus *Bacillus* sp. (Gupta *et al*., 2004).

Extracellular lipase ware produced by bacteria are influenced with the composition of growth medium, nutritional source and environmental condition (Anbu *et al*., 2011). The major factor for the expression of lipase activity has always been carbon, since lipases are by and large inducible enzymes and lipase was generally produced in the presence of a lipid source such as an oil or any other inducer, such as triacylglycerols, fatty acids, hydrolyzable esters, tweens, bile salts and glycerol (Gupta *et al*., 2004). For industrial process, the cost of raw material such as substrate should be have low price. The substrate for lipase production can utilize the slaughterhouse solid waste, one of the intestine which have high lipid content. The intestine storage fats from lipid metabolism, intestinal TAG synthesis can contribute to excessive accumulation of TAG in tissues (Yen *et al*., (2015). In addition, fat which melted from intestinal tissue could be a susbtrate for lipase production.

Besides, screening of extremophiles has become an important criterion for the production of highly active and stable lipases towards extreme environmental conditions. In previous research, *Bacillus* sp. SKII-5 which isolated from Dieng Plateau Creater, Central Java, Indonesia by Nur Tsurayya. Accordingly, had lipolytic activity using Tween80 as a substrate. *Bacillus* sp. SKII-5 included thermophiles bacteria. In addition, *Bacillus pumilus* which is isolated from the contaminated soil and it grown in *tributyrin* substrate that able to produce lipase with activity 3526.6 U/mg (Kumar *et al*., 2012). Futhermore, *Pseudomonas gessardii* which grown in goat tallow from slaughterhouses as a substrate, has lipase activity 1473 U/mg (Ramani *et al*., 2010). However, lipase whith higher activity are have great interest to be some experiment. There are no report on utilization of intestine cattle fat as a substrate for lipase production. Therefore, this study was focused on the production of lipase from *Bacillus* sp. SKII-5 using intestinal fat as a substrate, followed by purification.

MATERIAL AND METHOD

Isolation *Bacillus* sp. SKII-5

The lipase producing bacteria strain, was isolated from Dieng Plateau Creater, Central Java, Indonesia. The isolatation using nutrient agar medium that composition contain of peptone 0,5%, beef extract 0,3%, agar 1,5% and Sodium Chloride 0,5%, pH adjust to neutral, and was kept for 48 h. Futhermore, the colonies which grown picked up and placed to selective media which contain (g/l) peptone, 10; NaCl, 5; $CaCl_2.2H_2O$, 0.1; agar, 20; tween 80 (v/v), 10 mL and aquadest (v/v) (Kumar *et al*., 2012) to observed the hydrolytic activity. The lipolytic bacteria colony showing clear zone. *Bacillus* sp. SKII-5 was subcultured from pure stock and was grown on nutrien agar medium in Petri dishes every 2 weeks.

Fat as A Susbtrate and Its Characterization

The intestinal cattle fat in this study is a visceral organ which rich in fat. It was taken from Pegirian slaughterhouses Surabaya, Indonesia. The intestinal was melted in 110^{o}C to get the lipid source alone from the tissues. Fat which separated from tissue was placed in a sterile bottle and this was used as a susbtrate for acclimatization and fermentation media throught the production of lipase. Fat was weighed and hidrogen content analyzed carbon content by using Walkey Black method (AOAC, 1990) and nitrogen content by using Kjedhal method (Bvahan, 2004).

Lipase Production throught Submerged Fermentation

The bacteria *Bacillus* sp. SK II-5 cultures were grown in nutrient broth (pH 7.0) at 30^{o}C under shaking at 120 rpm, till a constant absorbance of 0.6 was attained at 600 nm in a spectrophotometer. The culture was revived thrice in nutrient broth medium before inoculating in to the acclimatization medium. The acclimatization medium which containt of intestinal fat, with 1% concentration was inoculated with inoculum at the rate of 10%(v/v). Then, bacterial culture were pick up and inoculating to production medium as much as 10% (v/v). The selected bacterial cultures were cultivated in 250 ml Erlenmeyer flasks containing 100 ml of an enrichment medium with the following composition of lipase production medium peptone (0.05%); KH_2PO_4 (0.1%); K_2HPO_4 (0.3%); Na_2SO_4 (0.2%); $MgSO_4.7H_2O$ (0.01%); gum arabic (0.1%); tween80 (0.1%); pH 7.0 (Sharma *et al*., 2014) with modification. Futhermore, fat were added with varied concentration of 0.11%, 0.21%, 0.31% and 0.42% (w/v). Culture of *Bacillus* sp. SKII-5 were incubated at 50^{o}C and under shaking at 120 rpm for 40 hours (Rajendran and Thangavelu, 2012). Bacterial growth was monitored and measured every 2 hours for 40 hours. Observations using UV-Vis spectrophotometer with a wavelength of 600 nm (Madigan *et al*., 2012).For lipase extraction, broth medium biomass were separated using filtration with paper Wattman No. 1, cells were separetd from the incubation or cultivation medium by centrifugation at 14,000 rpm for 5 minutes at 4°C (Ertugrul *et al*., 2007) and the supernatant was used a the source of extraxcellular enzyme to measure the activity of lipase.

Assay of Lipase Activity

Lipase activity was determined using p-nitrophenyl palmitate (pNPP) as a substrate. Lipase activity was done spectrophotometrically by following the hydrolysis of pNPP to pNP. Standard curve used is 4-nitrophenol (pNP). Color indicator that appears is yellow. Tris-HCl buffer pH 8 was made prior to the tests. Preparation of the substrate using a solution A (30 mg pNPP dissolved in 10 ml of isopropanol) with 90 ml of solution B (0.1 g Gum Arabic and 0.4 ml of Triton X-100 was dissolved in 50 mM Tris-HCl buffer pH 8.0), adding the solution was solution B to solution A, all the reagem are stirred until dissolved to a final volume of 100 ml (Ertugrul *et al*., 2007). Measurement of enzyme activity carried out by mixing 1.8 ml substrate solution and 0.2 ml of crude enzyme and incubated in a temperature of 30±1^{o}C for 30 minutes. Absorbance was measured using a UV-Vis spectrophotometer with a wavelength λ 410 nm. Activities per unit (U) is indicated in mol p-nitrophenol per minute under conditions of treatment (Sharma *et al*., 2014).

Partial Purification of Lipase

Lipase was partially purified using Ammonium Sulphate $(NH_4)_2SO_4$ with precipitation method according to the method Doung-Ly & Gabelli (2014). Crude Lipase is purified by adding of ammonium sulfate $(NH4)_2SO_4$ with a fraction of 15-30%, 30%-45%, 45%-60%, 60%-75% and 75-90%. Solid ammonium sulphate 2,46 gr was added to the 15 ml crude enzyme in 50 ml Beaker glass, with stirring to bring the saturation 15-30%, and the mixture were kept overnight. The precipitated protein was separated by centrifugation using a speed of 14,000 rpm, at 4°C for 10 minutes. The supernatant were added with $(NH4)_2SO_4$ to the next saturation until 90%, natant were added with buffer phospate to determine the lipase activity and protein content.

Protein Content and Molecular Weight

Protein content were determined using Bradford methode with Bovine Serum Albumin (BSA) as standart curve. Reagent made with 100 mg Coomassie Brilliant Blue G-250 dissolved in 50 ml of 95% ethanol, 100 ml of phosphoric acid 85% (w/v), added the destiled water and dilute until all dissolved and filtered using Whatman No.1 until the volume of 1 L Absorbance was measured with a spectrophotometer at λ 595 nm. Measurement

protein content of lipase carried out by reacting 0,1 ml Lipase and 5 ml Bradford reagent, homogenized using vortex 2500 rpm ± 1 minute. The solution was absorbance using spectrophotometer at λ 595 nm (Bradford, 2002). Polyacrylamide gel made consisting of 12% separating gel and 4% stacking gel (Burgess *et al*., 2002). Running polyacrylamide gel electrophoresis performed for 70 minutes with the power supply voltage of 100 volts. Results of running soaked with staining Coommasie Brilliant Blue R-250 for 20 minutes for the process of coloring the ribbon on the gel. Gel results put into immersion in hot water temperature of 150^{o}C to eliminate CBB which is remaining in the gel.

RESULT AND DISCUSSION

Lipase Production Using Intestinal Cattle Fat As A Susbtrate

Solid slaughterhouses waste had potential to be a substrate for lipase production, especially intestinal organ. In Pegirian slaughterhouses had 150 cattles that already cut everyday (Rahmawati, 2013). Intestinal orgas as much as 1 was melted in 110^{o}C, the lipid or fat which got is 348 g. Lipid metabolism during digestion influenced amount of fat storage in visceral organ (Drackley, 2000). About 85% fatty acid produced in rumen through fat digestion happened, then they toward to intestine and diffusion in mucosa epitel, esterified become triglycerides (TAG) subsequently in the intestine (Draper, 1982). The characterization of lipid which melted from intestinal fat are as follows. Carbon, nitrogen and hydrogen content is 71,82%, 2,11% and 13,82%, respectively. In previous report, the characterization goat tallow which utilizing as a substrate are as follow; carbon, hydrogen and nitrogen content 10.44 ± 0.1, 2.33 ± 0.13 and 0%, respectively (Ramani *et al*., 2010). In this research, among the composition of fat, carbon is the highest component. Macro-nutrient such as carbon, nitrogen and hydrogen was required by microbial during cultivated (Anderson and Jayaraman, 2003; Schaechter, 2006). Carbon used for membrane formation or cell wall, protoplasm and other product. Carbon contained in the substrate is oxidised to CO_2 during the metabolic activity (Pitchel, 2005; Byung and Gadd, 2008). Nitrogen and hydrogen are used by bacteria for protein synthesis, amino acids and nucleic acids in the protoplasm (Pitchel, 2005) as well as used for the growth and regulation of the cell (Moat *et al*., 2002).

Bacterial lipases are mostly extracellular and influenced by nutritional such as presence of lipids, the variation concentration of lipids were observed to got the most optimum concentration of substrate, with analyzing the lipase activity and protein content. Fig.1 shown during increasing the concentration from 0,11 to 0,31 g/100 ml of basal medium, lipase activity were decreasing from 2858,6 U/ml to 2256 U/ml, and increase to 2586,6 U/ml with concentration 0,42 g/100 ml of basal medium. Thus, the maximum lipase activity of approximately 2858,6 U/ml and the optimum concentration of substrate was 0,11% or 0,11 g/100 ml (w/v) of basal medium.

Lipase was producted in log (exponential) phase, that shown in Fig. 2. growth profile of *Bacillus* sp. SKII-5 in fermentation medium. Exponential phase characterized with hydrolyzing main carbon source intestinal fat by bacteria to produce extracellular lipase to got the nutritional during the cultivation process (Anderson and Jayaraman, 2003). Bacterial growth is proportional to the rate of bacterial death occurs in the stationary phase. This occurs due to the reduction of nutrients in the growth medium as well as the accumulation of byproducts of bacterial metabolism (Pommerville, 2014).

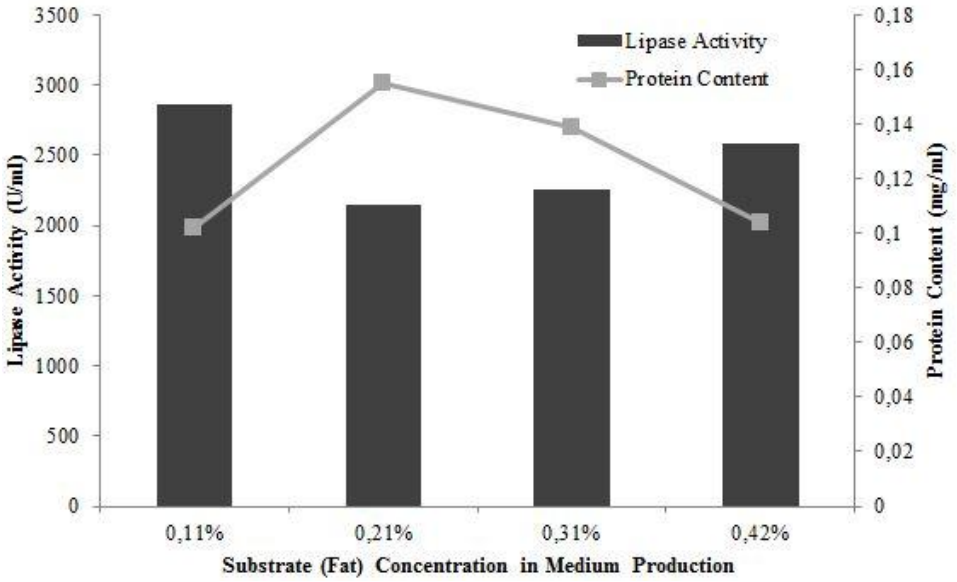

Figure 1. Activity and protein content of crude lipase.

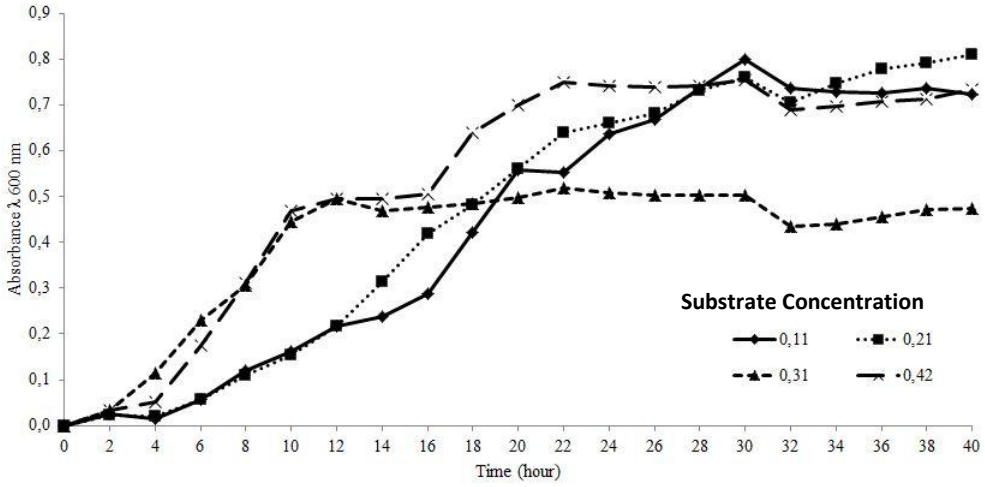

Figure 2. Growth curve of *Bacillus* sp. SKII-5 throught medium production.

Lipase Purification and Activity

Lipase purification needs to got purified protein, the principal is using ammoniu sulphate for saling out. Ammonium sulphate is one salt with high solubility. When salt is present, however, the anions and cations neutralize charges on the protein surface, preventing aggregation. As the salt concentration is increased even further, the surface of the protein will become so charged that once again, the protein molecules will aggregate (Duong-Ly et al., 2014). Each protein will settle on a different salt concentrations (Odutayo and Shazhko, 2013). Increase in lipase activity depends on the concentration of ammonium sulfate solution used Pabai *et al*., (1995).

Extraxcelluler lipase that produced by *Bacillus* sp. SKII-5 were partially purrified by ammonium sulphate at 60-75% saturation with highest activity and protein content is 1642,6 U/ml and 0,074 mg/ml, respectively (Figure 3). These results matches with the purification strategies followed by Kumar et. al. (2012) from *Bacillus* sp. RK31 60% saturation was effective concentration with 6.53 fold specific activities, besides Muthazhagan and Thangaraj (2014) reported that to precipitate lipase by ammonium sulphate, experiment was conducted at 20%, 40%, 60%, 80% and 90% saturation of ammonium sulphate salt. Results revealed that 60% saturation was proved to be effective for maximum specific activity of 60.97 U/mg with purification fold of 14%.

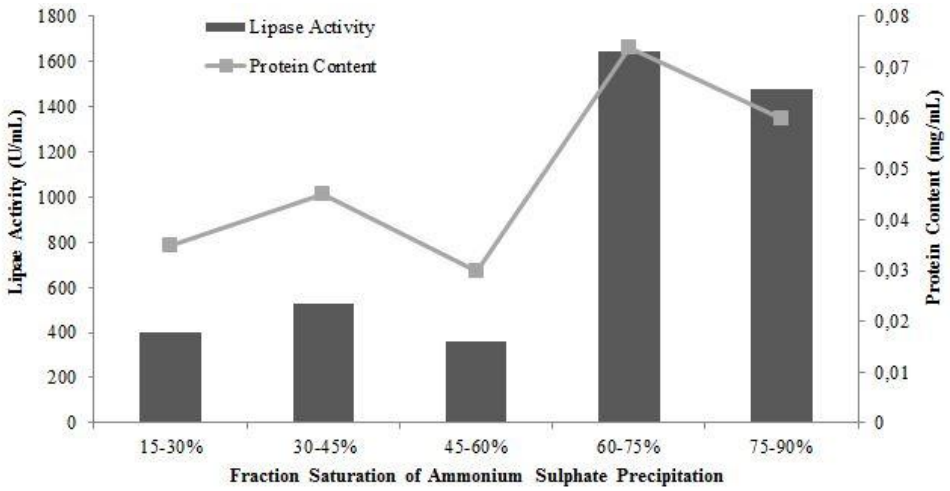

Figure 3. Activity and protein content of lipase which precipitation using ammonium sulphate.

Lipase activity was routinely estimated by employing the para-nitrophenyl palmitate (pNPP)1 assay described by

Winkler and Stuckmann Winkler *et al*., 1979). The basis of this assay protocol is the colorimetric estimation of para-nitrophenol (pNP) released as a result of enzymatic hydrolysis of pNPP at 410nm. One unit of enzyme activity is defined as the amount of enzyme liberating 1 lmol of p-nitrophenol per minute. pNPP added with buffer Tris-Cl and triton X-100. The adding Triton X-100 to the substrate solution to obtain a clear solution. Triton X-100 is a surfactant that causes dispersion of the fatty acids released due to enzymatic hydrolysis of pNPP (Gupta *et al*., 2002), resulting in a transparent solution (Figure 4.a). Lipase was breakd down ester bond in pNPP and released palmitate and p-nitrophenol (pNP) resulting in yellow solution (Figure 4.b-c).

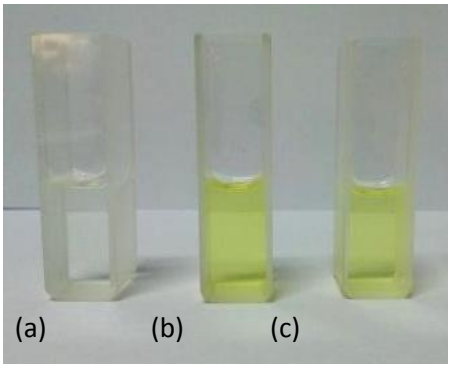

Figure 4. (a) pNPP solution (b) and (c) pNPP added with lipase and released pNP yellow solution.

Lipase Molecular Weight

Characteristics lipase was analyzed is molecular weight. The molecular weight of lipase was determined using SDS-PAGE. The molecular weight of lipase is between 19-75 kDa (Padilha and Silva, 2011). Based on Figure 5. The molecular weight of lipase was produced by *Bacillus* sp. SKII-5 is 43.3 kDa (Figure 5). This is matches by previous research that lipase produced by *Bacillus* sp. Has a molecular weight of 43 kDa (Shariff *et al*., 2011). In addition, Schmidt-Dannert (1996) also mentioned that lipase produced by *Bacillus thermocatenulatus* is 43 kDa. Lipase produced by *Bacillus* genus are classified into two subfamilies based on amino acid analysis and biochemical characters (Nthangenia *et al*., 2001). Lipase with a molecular weight of 19-20 kDa get into the

subfamily I.4. While lipase with a molecular weight of about 43 kDa get into the subfamily I.5. lipA gene encodes *Bacillus* lipase subfamily I.5 with characteristic length amino acid 388. The lipase with a molecular weight of about 43 kDa resulted from incubation temperature of 50^{o}C, so that they are thermostable (Shah *et al*., 2011; Shariff *et al*., 2011).

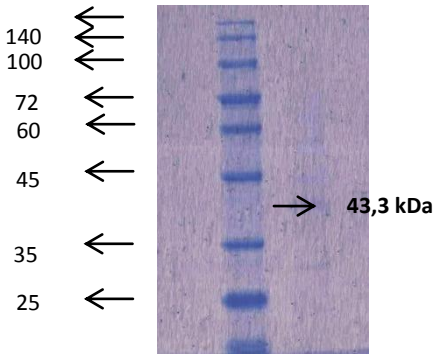

Figure 5. Molecular weight of lipase using SDS-PAGE.

CONCLUSION

The *Bacillus* sp. SKII-5 which isolated from Dieng Plateau Creater, Central Java has potential to produces inducible and extracellular lipase with highly activity is 2858,6 U/ml. Solid slaughterhouses waste is intestinal cattle fat could be utitizing as a substrate for lipase production, caused high lipid content. The enzyme was purified by ammonium sulphate precipitation, and the result revealed that 60-70% saturation was proved to be effective.

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Optimization of Potato (*Solanum tuberosum* L. cv. *Granola*) Callus Induction Using Different Explant Types With 2,4-D In Vitro

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ABSTRACT

Potato (*Solanum tuberosum* L. cv. *Granola*) is one of the horticulture plant which has prospect in the future. This plant can be developed with tissue culture method. The aim of this research is to obtain the data on the optimization of potato callus induction using different explant types with various of 2,4-D auxin hormone concentration. Design of this research is factorial completely randomized. The first factor is the plant growth hormone of 2,4-D with four treatment doses (0, 1, 2, 3 ppm) and the second factor is the types of explant such as roots, stems and leaves. Number of treatments are 12 with four replicates. Observation has been done for 42 days. The parameters are percentage of explants when form the callus, callus formation time, texture, color, fresh and dry biomass, surface area of the callus and percentage of the explants to form shoots. Result shows that all types of explants can form a callus except without using 2,4-D. Percentage of the stem explants which can form a callus higher than roots and leaves, texture and color have friable and white-greenish respectively. The stem explants with 3 ppm 2,4-D have showed highest callus fresh and dry biomass, surface area and percentage of callus forming shoots.

Keywords: Callus, Granola, optimization, potato, 2,4-*Dichlorofenoxyacetic acid*

INTRODUCTION

Potato (*Solanum tuberosum* L. cv. *Granola*) is horticulture plant which has edible tuber and good prospect in the future. This plant was classified in the solanaceae family. The potato plant is an herbaceous annual, which can propagated by planting pieces of tubers. Basically, the tuber contain carbohydrates, protein, vitamin C and B, potassium, phosphorus, and iron (Kumar *et al.*, 2014). Most farmers preserve potatoes of local varieties at home indigenously. Consequently, loss due to dehydration, pest attack and infection by pathogenic organisms is substantial (Parveen, 2011). The potato plants always are attacked by aphids (Setiawati *et al.*, 1993), root knot nematode (Hadisoeganda, 1993), thrips (Hubagyo, 1992), *Phytophthora infestans* (Suryaningsih, 1992) and various diseases which are classified according to their causal agents, such as bacteria, virus, fungus, flea beetles and nematodes (Duriat *et al.*, 1996). Some non-parasitic diseases or physiological diseases caused by physiological deficiencies or environmental factors are also noticeable.

Plant tissue culture have been developing as a modern techniques to improve the quality and quantity of vegetative propagated many plants. Kumar *et al.* (2014) explained that the techniques can be developed as a powerful tool for crop improvement. This technique was used to isolate the parts of the plant (the protoplasm of cells, tissues and organs) in sterile conditions so that these parts can multiply themselves and regenerate into complete plants. The techiniques can decrease and diseases free to good quality seeds and pathogen free planting materials are possible to produce through tissue culture. One of techniques in tissue culture is callus culture. The

callus culture can change the explants to amorphous formation for target material in genetic transformation and provide regeneration to shoots or planlets. Harahap (2011) described that the callus formation occurs from divide of the cells continuously and have a morphology amorphous. The callus was formed through three stages, namely induction, cell division and differentiation. Establishment of the callus was determined by explant, the composition of nutrients in the medium and the environmental factors. Source of explant was vascular, parenkim cambium, perisicle, cotyledon, leaves mesofil and provaskular tissue. Some studies reported that the potato is easily regenerated from different explants in MS medium supplemented with different auxin and cytokinin (Kumar *et al.,* 2014; Khatun *et al.*, 2003). Improvement of species through this technique depends on *in vitro* regeneration system and high regeneration frequency of plants from cells and tissues.

One of auxin to callus induction is 2,4-*Dichlorofenoxyacetic acid* (2,4-D). Besides, this hormone capable to induce callus, these hormones also play a role in inhibiting the formation of chlorophyll, roots and shoots formation (Kamal, 2011), plays a role in embryo-genesis, inhibits the formation of buds axilar and adventif, as well as induce callus if used in high concentrations (Oggema *et al.*, 2003). Chamandoosti (2013) stated that 2,4-D most effectively stimulates the formation of strong activity which was keep the process of cell differentiation, growth and organogenesis of callus. In this study, induction of potato (*S. tuberosum* cv. *Granola*) explant types using 2,4-D are early research to generate data and to provide raw material. The aim of this research is to obtain the data on the optimization of potato callus induction using different explant types with various concentration of 2,4-D. In addition, optimization of the technique of callus needs the best way to optimal results, effectively and quickly, as well as by low cost of optimization this technique of callus culture only with 2,4-D can be repressed and more effective.

MATERIAL AND METHODS

Plant material and callus induction

This study was carried out in a Laboratory of Plant Tissue Culture - YAHDI, Medan and Plant Physiology Laboratory at Departement of Biology, Faculty of Science and Technology, Airlangga University, Surabaya and was started from February – May 2014 and March – June 2016. The explants from sprouting tubers of potato (*S. tuberosum* cv. *Granola*) were used as sources. Samples of root which were the ends to be cut to the size of 1 cm. Samples of stem that is part of the first segment of the base until the third segment and samples of leaf were the second leaf from the tip, measuring 1 cm. All the explants were washed thoroughly under tap water for 15 minutes. The explants were further treated with 2-3 drops of bactericide and fungicide for 10-15 minutes followed by thorough washing with distilled water. The explants were then surface sterilized with 5 % (w/v) $HgCl_2$ for 5 minutes under laminar airflow. After that, explants were washed 4-5 times with sterile double distilled water to remove traces of mercuric chloride. All culture bottles contained 25 ml of agarized medium (MS added with 30 mgL^{-1} sucrose and 2,4-D). All media contained 30 gL^{-1} sucrose and pH was adjusted to 5.8 with 1.0 N HCl or 1.0 N NaOH before adding 7 gL^{-1} agar and autoclaving at 121 ^{0}C for 20 min. Culture bottles were placed in a culture room at 25 ± 2 ^{0}C and exposed in the dark room. Callus initiation was observed within 7 days after inoculation for 42 days. Subsequently, callus were subcultured every 2 weeks.

Growth dynamics of callus

Growth of callus was established in this study. Each subculture (1st to 3rd subculture) provide data of the callus fresh biomass. Percentage (%) and time (day) of callus formation, callus color, callus texture, callus necrotic were determined and recorded weekly for 42 days. Callus induction frequency (CIF) was determine according to Htwe *et al*. (2011):

CIF (%) = Number of explants producing callus x 100 / total number of explants

Effect of Explant types and 2,4-D

Explant types were induced from root, stem and leaf of *S. tuberosum* cv. *Granola.* Explants were cultured in callus induction medium containing MS basal media supplemented with various 2,4-D (0, 1, 2, 3 ppm). The surface area (cm^2), fresh and dry biomass (g) and percentage of callus to form shoots (%) were observed and recorded weekly for 42 days. Determination of surface area, fresh and dry biomass of callus was established in this study. The callus was separated from the medium through a stainless steel sieve. Then, the fresh biomass was measured after blotting away. The dry biomass was weighted after drying at 60^0C for 48 h.

Experimental Design and Statistical analysis

This study used Completely Randomized Design (CRD). The callus induction experiments were conducted with four replicates. Data was obtained from all experiments which was presented as the mean ± standar eror (SE). The data was analyzed using SPSS V. 17 and the differences between the means were compared by Duncans's multiple range tests at the 0.05 level.

RESULTS AND DISCUSSION

Growth dynamics of callus tissue

Percentage of explants forming callus

In all the media, the maximum percentage of explants forming callus was observed on the stem explants (100%) in the MS basal medium added with each 1, 2, 3 ppm 2,4-D, but percentage on the leaf are 50%. Meanwhile, percentage on the root are 100%, 75% and 50% in 2, 3, and 1 ppm 2,4-D respectively (Table 1). According to Raghavan (2004) that provide 2,4-D on the explant can affect the formation of callus. There is a relation between auxin (2,4-D) and the occurrence of cell abnormality. The occurrence of cell abnormality was caused due to disruption of DNA replication during mitotic division. Oggema *et al..* (2007) explained that the phenomena can be induced by auxin to form the callus if used in high concentrations. Michiba *et al.*. (2001) also reported that 2,4-D in high concentration caused damage to the cells in their study of *Doritaenopsis* cell suspension. Each explant has different response when induced by hormones and influence towards the process of the callus formation. This is due to biological conditions of the explant. In medium supplemented with hormone, the cell of callus contacts with the media which pushed into meristematic tissue and conducted to close the wound. Types of young explant (meristematic) more easily to form a callus than old callus. The older of the explant age would hard to form callus because has lignification (hardening). However, the success of in vitro cultures depends on the harmonious combinations of nutritional constituents and plant growth regulators (Htwe *et al.*, 2011). Pandey *et al..* (1994) also added that the success of this technique largely depends on the nutritional media, plant growth hormones, genotypes and the interaction genotype.

Time of callus formation

Based on the observations, the explants from stem rapidly to form callus (7 days), followed by root (8 days) and leaf (11 days) in MS basal medium supplemented with 3 ppm 2,4-D (Fig. 1). The formation process was began at lump on the explants. The lump was caused by wound at the explants and this way as a response to form a callus. In contrast, MS basal medium without hormone did not respond to callus induction. In this tudy, the callus of the stem explants more survive in medium with 1, 2 and 3 ppm 2,4-D (Fig. 2). The callus can survive to 35 days after appear a callus on 7 days. In this study has observed that survival time of the callus on the stem higher than root and leaf. Even, the callus from leaf more sensitive.

Color and texture of callus

Callus color MS basal medium supplemented with 1, 2, 3 ppm 2,4-D are white, white-greenish and white-greenish respectively from stem explants, but the callus color from root and leaf explants are pale-white or pale-brownish (Table 1). The callus of the roots and the stems more friable, except from leaves (Table 1) (Fig. 3). Ghareeb *et al.* (2009) explained that the different concentration of 2,4-D led to callus induction with distinctive colors and Roostika *et al.* (2012) in their research reported that auxine of 2,4-D induced compact and greenish calli rather than friable and yellowish callus which were usually observed in the embryogenic callus. Some researcher reported that the potato is easily regenerated from different explants in MS medium supplemented with different auxin and cytokinin (Kumar *et al.,* 2014; Khatun *et al.*, 2003). Khatun *et al*. (2003) explained that various combination and concentration of auxins and cytokinins were effective for callus induction. Theoretically, equal amount of auxin and cytokinin promotes callusing, but in practice it differs to a good extend may be due to the variation in endogenous level of phytohormones. Lizawati (2012) reported that the combination 2,4-D and TDZ produced the time appearing of callus fastest compared to other treatments. Kumar *et al.* (2014) also reported that the best growth of callus from both the cultivars was observed on Murashige and Skoog (MS) in media containing 3.0 mgL^{-1} of 2,4-D and 1.0 mg L^{-1} of kinetin. Generally, the use of strong activity auxin (2,4-D) combined with generally low concentrations of cytokinin can induce friable callus. In contrast, this study only using 2,4-D has found friable callus and embryogenic callus (Fig. 5). Furthermore, the callus which friable, soft and white-greenish usually has a higher regeneration ability to form somatic embryos than compact callus. Besides, Kumar *et al.* (2014) has reported that the interaction of both hormones had significant result on callus induction but low concentration of 2,4-D with high concentration of kinetin was found ineffective. Even, Tan *et al..*, [20], treatment 2,4-D alone at 2 mgL^{-1} to *C.asiatica* had the high percentage of calli.

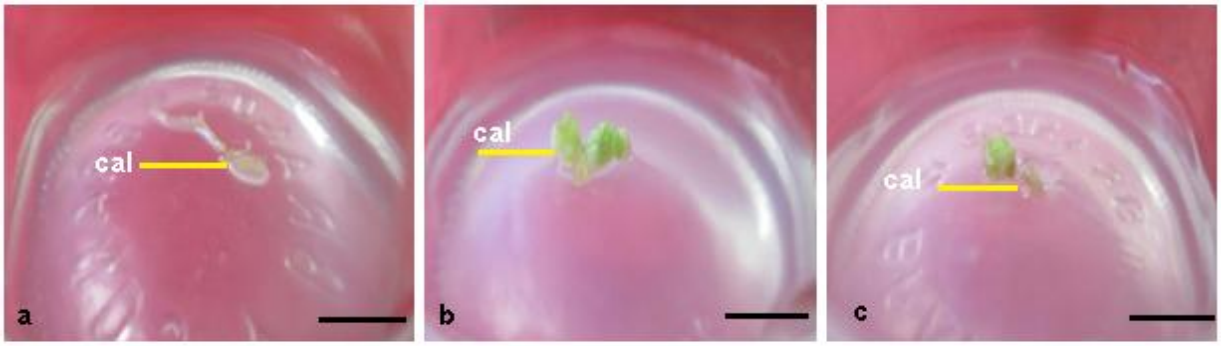

Figure 1. First time of callus formation from explants in MS basal medium supplemented with 3 ppm 2,4-D; a) root 8 days, b) stem 7 days, c) 11 days. (Bar=1cm)

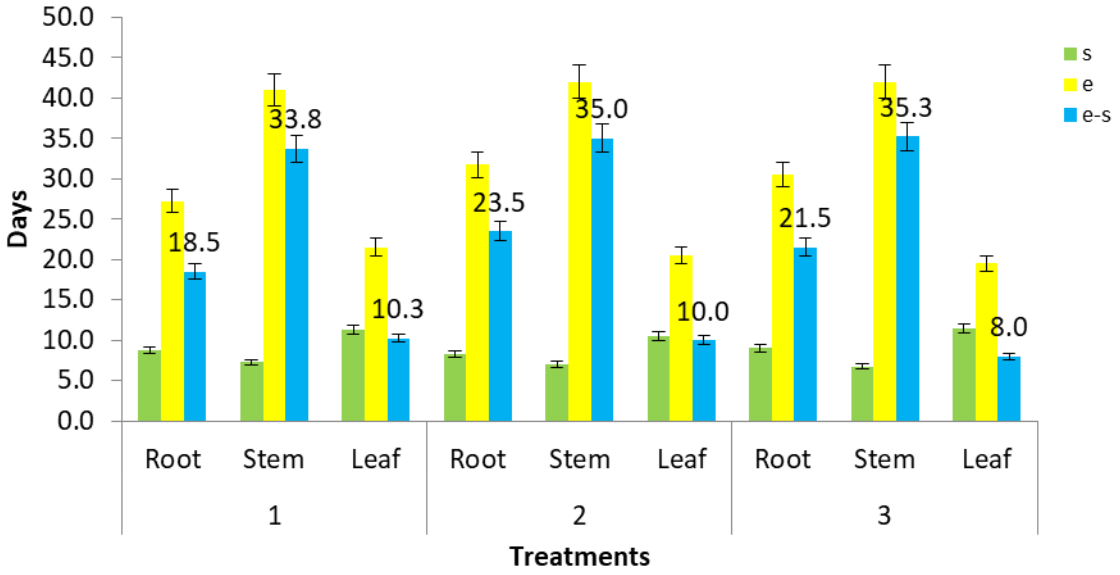

Figure 2. Survival of callus from the root, stem and leaf explants of *S. tuberosum* cv. *Granola* inoculated on the MS basal medium supplemented with various of 2,4-D for 42 days. Data are presented as the means ± SE (n=4); s-start; e-end; e-s–end-start

Effect of explant types and 2,4-D

The effect of different supplementations of 2,4-D on the callus induction of three explants was determined (Table 1). In the same morphological appearance, the maintenance and multiplication of the callus were conducted. This study has examined the developmental stage of the transformational study and the optimum stage for sub-culturing (Fig. 4). Variations in response for the callus growth on the fresh biomass was clearly observed when the mean values of fresh biomass were compared (Table 1). The highest of fresh biomass can be found on the stem explants in MS basal medium added with 3 ppm 2,4-D.

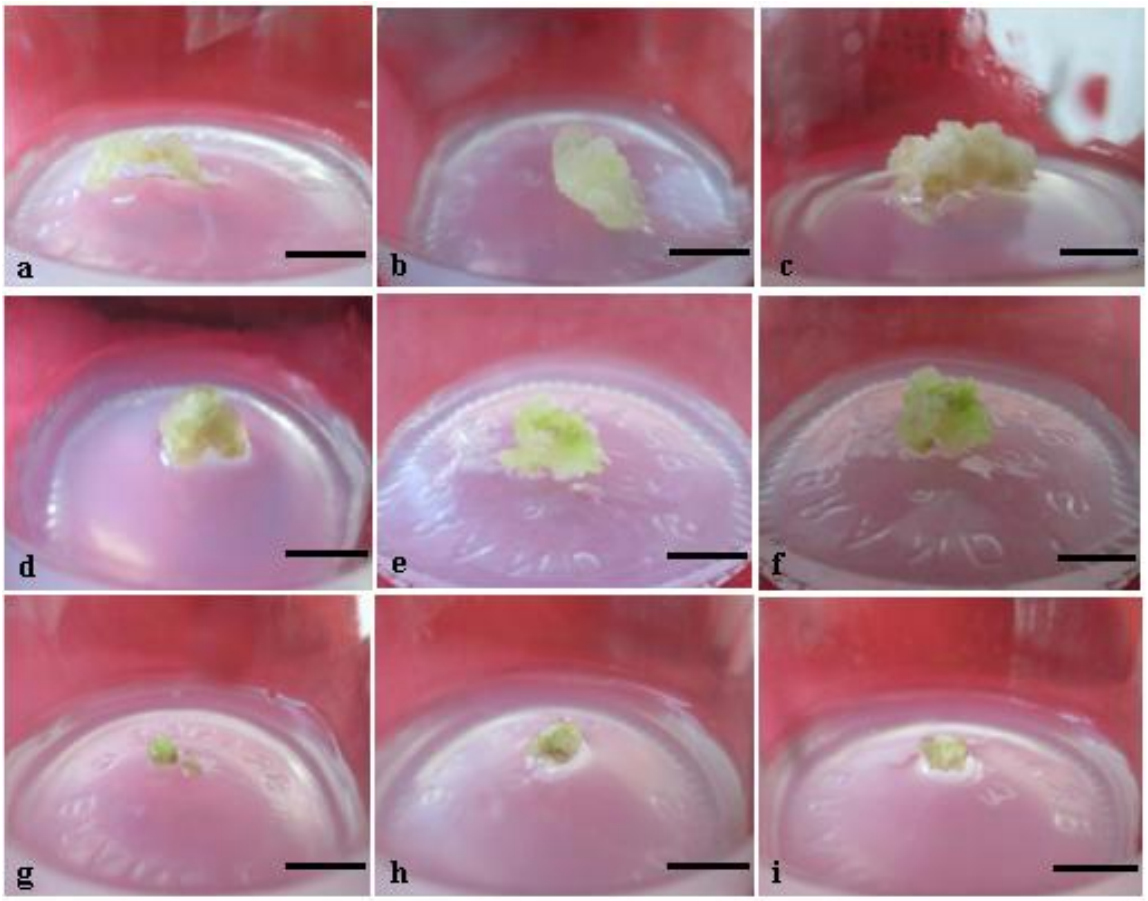

Figure 3. The color and texture of callus at 2nd subculture: a-c. roots in 1, 2, 3 ppm 2,4-D; d-f. stems in 1, 2, 3 ppm 2,4-D; g-i. leaves in 1, 2, 3 ppm 2,4-D. (Bar=1 cm).

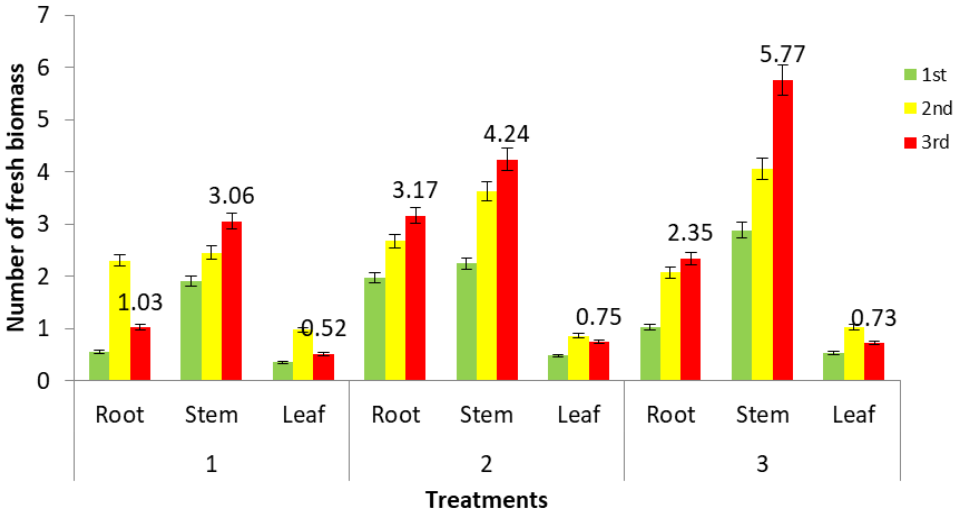

Figure 4. Subculture of fresh callus biomass from the root, stem and leaf explants of *S. tuberosum* cv. *Granola* inoculated on the MS basal medium supplemented with various of 2,4-D for 42 days. Data are presented as the means ± SE (n=4); 1st sc – 1st subculture; 2nd sc - 2nd subculture; 3rd sc – 3rd subculture.

The observation has showed significant differences of fresh biomass (fb) and dry biomass (db) among the callus. Apparently, the highest fresh biomass (5.77 g) and dry biomass (0.63 g) were found on the stem explants in MS basal medium supplemented with 3 ppm 2,4-D (Table 1), followed by in concentration of 1 and 2 ppm 2,4-D showed fresh biomass are 3.06 g and 4.24 g and dry biomass 0.35 g and 0.45 g. Meanwhile, the fresh weight and dry weight of root explants are 1.03 g fb and 0.09 g db in 1 ppm 2,4-D, 3.17 g fb and 0.32 g db in 2 ppm 2,4-D, 2.35 g fb and 0.22 g db in 3 ppm 2,4-D. For leaf explants are 0.52 g fb and 0.04 g db in 1 ppm 2,4-D, 0.75 g fb and 0.06 g db in 2 ppm 2,4-D, 0.73 g fb and 0.06 g db in 3 ppm 2,4-D. The surface area of callus from the stem explants in 3 ppm 2,4-D (6.21 cm^2) higher than the others . The callus has low surface area can be on the leaf in 1 ppm 2,4-D (0.84 cm^2).

Table 1. The callus induction from the root, stem and leaf explants of *S. tuberosum* cv. *Granola* inoculated on the MS basal medium supplemented with various of 2,4-D for 42 days.

Treatments		Explant forming callus (%)	Callus forming shoots (%)	Surface area (cm^2) (Mean±SD)	Fresh Biomass (g) (Mean±SD)	Dry biomass (g) (Mean±SD)	Callus color	Callus texture
2,4-D (ppm)	Explants							
0	Root	NC	NC	NC	NC	NC	NC	NC
	Stem	NC	NC	NC	NC	NC	NC	NC
	Leaf	NC	NC	NC	NC	NC	NC	NC
1	Root	50	0	1.58±0.44a	1.03±0.35a	0.09±0.03a	pale-white	Friable
	Stem	100	0	4.31±0.67c	3.06±0.85bc	0.35±0.11bc	white-brownish	Friable
	Leaf	50	0	0.84±0.72a	0.52±0.07a	0.04±0.01a	white-greenish	Compact
2	Root	100	25	4.34±1.38c	3.17±1.03bc	0.32±0.10bc	pale-white	Friable
	Stem	100	25	5.04±0.71cd	4.24±0.76c	0.45±0.10c	white-greenish	Friable
	Leaf	50	0	0.91±0.18a	0.75±0.26a	0.06±0.02a	pale-white	Compact
3	Root	75	0	2.64±0.38b	2.35±0.51b	0.22±0.03b	pale-brownish	Friable
	Stem	100	25	6.21±0.69d	5.77±0.52d	0.63±0.10d	white-greenish	Friable
	Leaf	50	0	1.16±0.74a	0.73±0.58a	0.06±0.05a	pale-white	Compact

Data (surface area, fresh biomass and dry biomass) represents mean values (n=4). Mean separation within column by Duncan's multiple range test at $P \leq 0.05$; SD-Standard deviation; NC-No Callus formed.

Different types of explant greatly affect the callus induction process especially on the biomass and the surface area of callus. In this study, the different explant such as root and stem higher than leaf to form callus. The stem explant was faster than the root. This is possible in the stem has endogenous auxin concentration was lower than the root, therefore the stem explant capable to form callus faster when was gave the concentration of 3 ppm 2,4-D. This phenomena was evidenced by the callus formation time of stem explant on 7 days, root on 8 days and leaf on 11 days after inoculation (Fig 1). The results are statistically data analysis shows that differences between the explant on the surface area, fresh and dry biomass of callus. The biomass and the surface area of the stem highest from the others.

The callus induction to 3rd subculture shows the callus from stem explants, as well as the embryogenic and non-embryogenic callus (Fig. 5A and 5B). Zhang *et al.* (1996) reported in their study that induction of embryogenic callus in rice was considered the most critical step. Van *et al.*. (1990) added that characteristic of the embryogenic

callus usually light yellow to white in color, as well as dry and nodular. Meanwhile, the non-embryogenic callus appeared to light yellow, watery and non-nodular. In this present study, the MS basal medium containing 2 ppm 2,4-D on the stem and the root was successfully used to induction of embryogenic callus. The embryogenic callus which white-greenish in color, dry, nodular and friable has transformed to shoot. Efficiency of plant regeneration system needs to be fixed in the embryogenic callus as the target tissue in a reliable genetic modification system. In this observation was found the embryogenic callus which showed green spot and the callus transform the planlet (Fig. 6). Percentage of callus forming shoot are 25 % from the stem and the root in (Table 1). In addition, the differences were also observed between the explants. This investigation also was found the non-embryogenic callus, as well as necrotic callus. The callus which appear brownish in color can potential to form necrotic. This callus has found in 3rd subculture in 2 ppm 2,4-D. Roostika *et al.* (2012) reported the phenomenon of browning could be observed from the highest concentration supposed to caused cells necrotic since it is a herbicide agent. Simultaneously, some callus began to turn pale-brownish and eventually died.

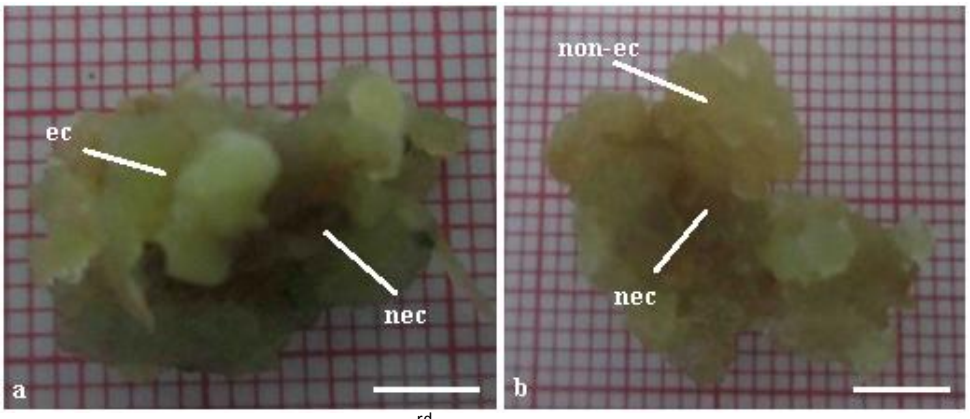

Figure 5. Induction of callus from stem explants after 3rd subculture: a. embryogenic callus (ec); b. non-embryogenic callus (non-ec); nec-necrosis callus. (Bar=0.5 cm).

Hoque *et al.*. (2007) in their study at rice tissue culture, described that combination of medium and suitable genotypes should be used to increase the plant regeneration frequency. These variations would be due to the differences in the concentrations and components of endogenous phytohormones, as well as the differences in their responsiveness to 2,4-D. The frequency of callus higher than regeneration frequency. Huan *et al.*. (2004) added that the callus exhibited high regenerative potential and the capacity to proliferate in later subcultures. The subculture can initiate newly medium for callus and will have a minimal proliferation rate, but this way may early lead to the death phase.

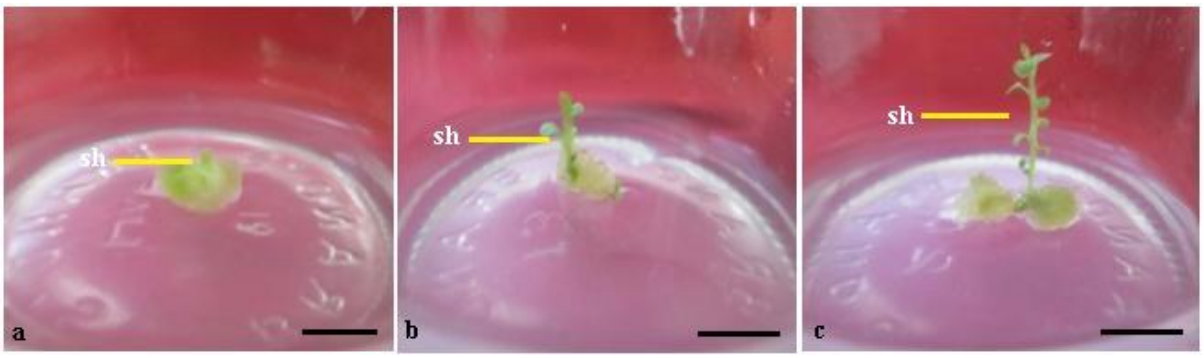

Figure 6. Shoot formation of callus from the stem explants in 2 ppm 2,4-D: a. initial shoot after 28 days; b-c. growth of shoot after 35 days and 42 days. (Bar=1cm)

Many authors emphasized the 2,4-D as an essential element for callus formation and proliferation in many plants (Kasi and Sumaryono, 2008; Winarto *et al.*, 2009; Roostika *et al.,* 2012). The data presented in this study suggested a regeneration scheme for *S. tuberosum* cv. *Granola*. In our point of view, after the induction phase of callus, selecting the most appropriate medium and judging the genotype capacity of plant for callus proliferation and regeneration. It can be facilitate to its regenerability for genetic transformation using callus as target material, as well as advantages of callus culture is to study of metabolism and cell differentiation, morphogenesis of cell, variation of genetic transformation, somaklonal as well as the production of secondary metabolites. In addition, callus culture also done to clone plant reproduction through the formation of organs and embryos, genetic variants of regeneration, as a source for cryopreservation and bio-transformation.

CONCLUSION

Optimization of potato (*Solanum tuberosum* cv. *Granola*) callus induction using different explant types with various of 2,4-D auxin hormone concentration shows that all types of explants can form a callus except except without using 2,4-D. Percentage of stem explants which can form a callus higher than roots, texture and color have friable and white-greenish respectively. Stem explants with 3 ppm 2,4-D have showed highest callus fresh and dry biomass, surface area of the callus and form shoots.

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Renewable Innovation of Lime Leaves Extract and Refined Eucalyptus Oil Combination Formula Based on Hair Steamer as a Quick Treatment for *Pediculosis capitis*

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ABSTRACT

Pediculosis capitis or well-known as head lice disease often happen in the world populations, especially children. Pediculosis capitis causes various problems such as scalp abscess, itchiness, low sleep quality, embarrassment and low self-esteem. The patients of Pediculosis capitis usually used chemical products (drugs) that can cause side effects for body health if used continuously. The aim of this study was to find a natural alternative treatment for Pediculosis capitis, using hair steamer technology. Lime leaves were extracted by maceration method for produce limonoids. The yields of extract then being mixed with eucalyptus essential oil. These herbal materials were evaporated using the hair steamer. The ratio of volume (lime leaf extract : eucalyptus essential oil) were 1:1, 1:2, and 2:1. The results showed that higher volume of lime leaf extract (that contain limonoids) could kill more head lice (volume ratio 2:1) which the mortality rate on eggs (nits) was 90% and adults was 95%.

INTRODUCTION

Pediculus humanus capitis included in Insecta, are ectoparasite on a human head that causes Pediculosis capitis. Pediculosis capitis can cause various effects on the patient, such as reduced quality of sleep due to itching, social stigma, shame, and low self-esteem (Stone, 2007). Itching and burning sensation in Pediculosis capitis result an irritation of the scalp due to scratching when itchy scalp. These parasites attack up to 40% of the world population, which is the highest the prevalence in children aged less than 12 years old (Pilger, 2010).

Pediculosis capitis, in general, has been treated using a spesific comb (lice comb) and anti-head lice drugs. The usage of lice comb can not eradicate head lice and larvae as a whole, but only reduces the number of lice. On the other hand, the use of anti-head lice has side effects, because it contains chemical compounds that cause skin irritation, dizziness, numbness, headache, nausea, and vomiting. Based on these facts, the best step to the handling of Pediculosis capitis is required.

Lime and eucalyptus have bioactive compounds include a natural pesticide. Botanical pesticides have is safer because of its made from natural ingredients. The previous research by Rina Murdani (2014), explained that the plant pesticide will not induce insect-resistant. Lime leaf extract contains substances called limonoids. Limonoids can be used as insecticides that inhibit and reduce the growth of insect, antibacterial and antifungal. This natural larvicides also kill the larvae of insects. Eucalyptus contains essential oils. Koul *et al.* (2008) stated that the biological activity of essential oils are repellent to insects. It is toxic and respiratory toxins as well. It also reduces appetite, inhibit eggs laying, inhibit growth, lowered fertility, and kill the insect vectors.

LITERATURE REVIEW

Pediculosis capitis

Pediculosis capitis is a scalp infection caused by head lice (*Pediculus humanus* var. *capitis*). *Pediculus humanus* var. *capitis* are ectoparasite head lice that infected human in general. These head lice can be infected by direct contact with a patient or indirect contact by using or borrowing patient's hair comb or hat. This infection generally happens because of bad or low personal hygiene of patient. The life cycle of *Pediculus humanus capitis* has three stages: nits (eggs), nymphs and adults (imago) (Laily, 2012). It needs 7-14 days for developing in all stages (Wijayanti, 2007).

Based on many cases in society, Pediculosis capitis has general symptoms such as itchiness on occipital and temporal section and spreading on all parts of the head scalp. Itchiness will make the patient scratching their head then caused erosion, crust excoriation and secondary infection (lesion). The secondary infection will cause hair agglomerate, bad smell and regional (occipital and temporal) lymph gland swelling (Handoko, 2007). Pediculosis capitis in children can cause many impacts as decreasing sleep quality, social stigma, embarrassment, and low self-esteem (Stone, 2008).

At this time, some treatments have been done to overcome Pediculosis capitis which using lice comb and anti-head lice drugs. The use of anti-head lice drugs can cause irritation on the scalp because of chemical contains such as lindane. Lindane cause burning sensations on the scalp, itchiness, even rash. Lindane was classified as moderately hazardous chemicals (Pilger *et al*., 2010).

Lime Leaves *(Citrus aurantifolia)*

Lime (*Citrus aurantifolia*) are a plant that contains natural pesticide materials called limonoids. Limonoids are a terpenoid compound (triterpenes) serves as larvacide (Ferguson, 2002). Based on research by Prijadi *et al*. (2014), the results showed that the mortality rate of *Aedes sp.* larvae was 67%. It can be used as a natural larvicide and effective to inhibit the larval development of *Aedes sp*. The previous research by Reni (2008) showed that the lime leaves have high efficacy as a repellent for mosquitos up to 87.97%. The results of other research by Rina Murdani (2014) demonstrated that the higher concentration of lime leaf extracts has higher mortality rate of *Aedes aegypti* larvae.

Eucalyptus Oil *(Melaleuca leucadendra)*

Eucalyptus oil is used more in pharmacy industries. Eucalyptus oil is used to cure stomachache and internal digestive tract, catching colds for adults and children, and as an external drug for a headache, rheumatics, cramped feet, and joints illness. Moreover, eucalyptus oil can be used as an insecticide. The essential oil of eucalyptus has efficacy as an insecticide or larvicide. Essential oil can be in liquid or solid form, volatile, and have various boiling points. Essential oil is produced by the plant in the leaves, roots, barks, stems, seeds, flowers, and fruits (Sastrohamidjojo, 2004). In eucalyptus, this essential oil obtained by extraction of the leaves.

Hair Steamer

Hair steamer is one of equipment that often used in beauty salons. Hair steamer usually used for cleansing the scalp after giving the vitamins or proteins for hair (hair mask treatment or hair cream bath). This equipment works by evaporate the water in the tube compartment. The steam will open up the hair follicle and hair shaft cuticle, allowing the drug gets into the pores of the scalp. Commonly, hair steamer used for 10-15 minutes.

MATERIALS AND METHODS

This was an experimental research using combination of lime leaf extract and eucalyptus oil to eradicate *Pediculus humanus capitis*. The sample used the head lice (*Pediculus humanus capitis*), its adults (imago) and eggs (nits). The equipment used were knives, blender, analytical balance, beakers, Erlenmeyer flask (size of 2 liters), stirring rod, aluminum foil, rubber straps, bottles, funnels, filter paper, measuring cups, spoons, hair steamer, lice comb, white paper, plastics, jars, Petri dishes, and microscopes.

Extraction Process dan Test Solutions

Extraction (Maceration) of Lime Leaves

The extraction of lime leaves has done in Integrated Laboratory, Diponegoro University, Semarang. Lime leaves were crushed by a blender to obtain powdered lime leaves. Powdered lime leaves then soaked in 96% ethanol with the ratio of 1: 2 (Yenie, *et al*., 2013), then left for one week in a sealed container. After 1 week, the solution then filtered using filter paper and funnel. The solution then separated from the extract and the solvent by evaporation using a rotary evaporator and concentrated with a water bath.

Lime Leaf Extract (Liebermann-Burchard) Test

Extracts that have been obtained then tested to determine whether it contains limonoids or not. This qualitative test conducted using Liebermann-Burchard reagent (0.1 mL of concentrated sulfuric acid and 0.5 mL of acetic acid anhydride). When the extracts are positive containing limonoids, the color turns red, pink, or purple. This test was performed at the Laboratory of Organic Chemistry, Faculty of Science and Mathematics, Diponegoro University.

Mixing Lime Leaf Extract with Eucalyptus Refined Essential Oil

Extraction of 21.5 kg lime leaves yield 418 mL extracts. The lime leaf extract and eucalyptus essential oil are mixed with different proportions. The volume ratio that used in this study (lime leaf extract and eucalyptus essential oil) was 1:1 (104 mL:104 mL), 1:2 (104 mL:208 mL) and 2:1 (208 mL:104 mL).

Sampling *Pediculus humanus capitis* and Samples In Vitro Incubation

There were 60 of adult lice and 30 louse eggs. All samples obtained from 8 children (6-12 years old), residents of Kalikayen, East Ungaran, Semarang. Samples were taken by using a lice comb, then placed into plastics. Furthermore, adult lice and eggs were transferred into 6 different jars. Each jar consists of 10 head lice and 5 eggs. The jar closed and only perforated on the lid of the jar as the air ventilation. In addition, the hair from respondents was also put into the jar as its medium.

Testing the Solution on *Pediculus humanus capitis*

The solution test on pediculicidal activity has been done at the Laboratory of Ecology and Biosystematics, Faculty of Science and Mathematics, Diponegoro University. The solution tested on samples performed by evaporating the lime leaf extract that has been mixed with eucalyptus essential oils. Each experiment, consists of 10 adult lice and 5 eggs. The extract evaporated using a hair steamer and the steam entered through the hole in the lid of the jar. The test conditions also made in the dark by using a trash bag, so that steam trapped and did not spread, its only entered into the jar. Each solution evaporated for 20 minutes. Experiments were repeated twice for each solution (volume ratio). The adult lice and the eggs then observed using a microscope after 60 minutes. Criteria of the death of head lice were defined as the absence of movement of limbs and gut, with or without stimulation using forceps. Louse eggs with closed operculum and nymphs inside were the criterion for embryo

mortality (abortive eggs) (Di Campli, *et al*., 2012). The results (the number of death adult lice and eggs) recorded then compared to see which volume ratio more effective to kill head lice.

RESULTS AND DISCUSSIONS

Maceration from 21.5 kg of lime leaves and 43 liters of ethanol 96% produced ± 418 mL of crude extract. The extract tested qualitatively by the Liebermann-Burchard test (0.1 mL of concentrated sulfuric acid and 0.5 mL of acetic acid anhydride). The result was positive contains limonoid (indicated by the onset of blue-purplish color on the extract).

Table 1. Treatment Results of Adult Lice (Mortality Based on Volume Ratio of the Solution).

Lime Leaf Extract (mL)	Eucalyptus Essential Oil (mL)	Volume Ratio	Total of Adult Head Lice	Total of Death Lice	Mortality Rate
50 mL	100 mL	1:2	20	15	75%
50 mL	50 mL	1:1	20	17	85%
100 mL	50 mL	2:1	20	19	95%

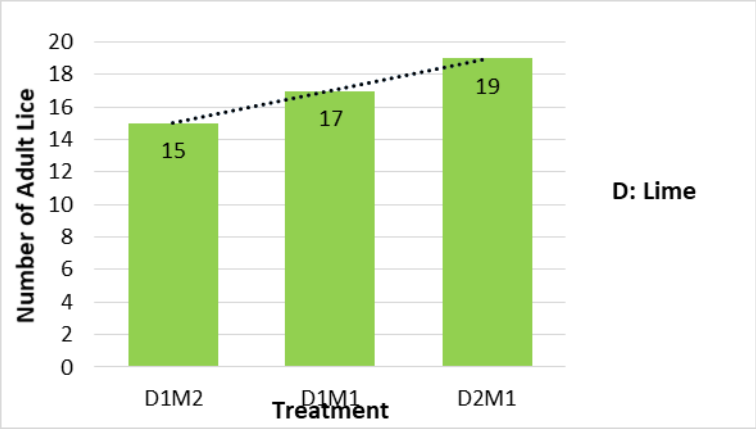


Figure 1. Treatment Correlation with Adult Lice Mortality.

Table 2. Treatment Results of Louse Eggs (Mortality Based on Volume Ratio of the Solution)

Lime Leaf Extract (mL)	Eucalyptus Essential Oil (mL)	Volume Ratio	Total of Louse Eggs	Total of Death Lice	Mortality Rate
50 mL	100 mL	1:2	10	4	40%
50 mL	50 mL	1:1	10	6	60%
100 mL	50 mL	2:1	10	9	90%

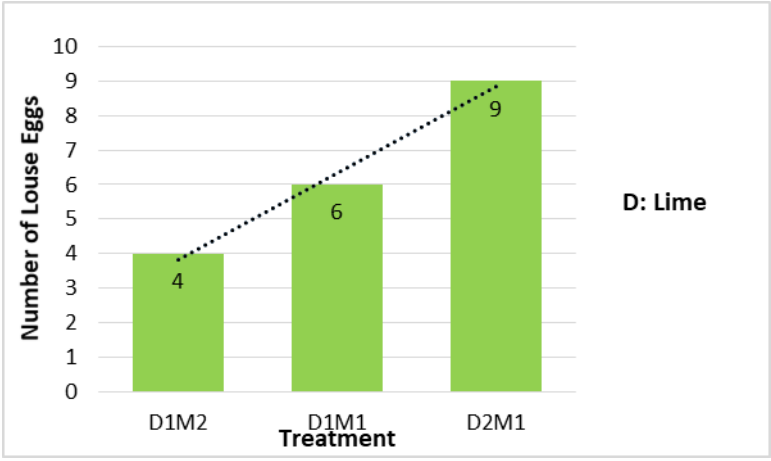
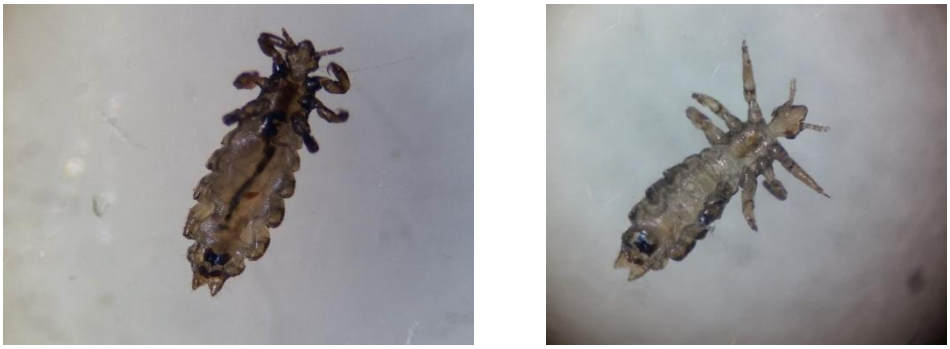


Figure 2. Treatment Correlation with Louse Eggs Mortality.



(a) **(b)**

Figure 3. Adult louse dead after 20 min of treatment with the solutions, showing the absence of gut and limbs movement (a); in contrast to adult louse that still alive (b).

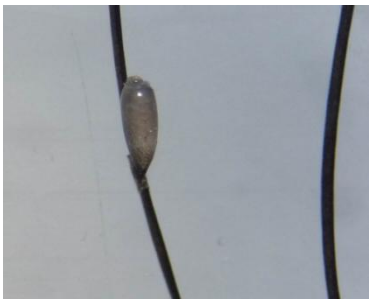


Figure 4. Louse egg dead after 20 min of treatment with the solutions (lime leaf extract and eucalyptus essential oil), showing the closed operculum.

The result (Table 1; Fig. 1) showed that the number of death of adult head lice each comparison (volume ratio) were different. At a ratio of 1:2, from 20 adult lice, there were 15 dead. At a ratio of 1:1, 17 of 20 adult lice were dead and at the ratio of 2:1, there were 19 dead. The highest number of death adult head lice at a ratio of 2:1, in which the mortality rate reached 95%. The dead adult lice characterized by the absence of gut and limbs movements (Fig. 3).

Table 2 and Figure 2 showed that the number of death of louse eggs each comparison (volume ratio) were different. At a ratio of 1:2, from 10 louse eggs (nits), there were 4 dead. At a ratio of 1:1, there were 6 of 10 louse eggs dead and at a ratio of 2:1, there were 9 dead. The highest number of death of louse eggs was at a ratio of 2: 1, with the mortality rate reached 90%. The dead louse eggs characterized by closed operculum (Fig. 4).

The results showed that more volume of lime leaf extract on a mixture of the test solution resulted in higher death rates. The lime leaf extracts were better for deadly head lice infestation. This can be caused by several factors: a) lime leaves contain limonoids, a toxic compound can cause a bitter taste so that lice can die; b) lime leaves that used are old leaves (dark green) in which the contain more limonoids than young lime leaves; c) lime leaves that used in this research was still fresh and picked from the tree, so the leaves contain more limonoids (Oktavia, 2013). Unlike the eucalyptus essential oil, which acts directly as a pesticide, contains cineol that causes the pungent aroma, so the lice do not like it. The scent from the eucalyptus oil was less influential in killing head lice than the limonoids of the lime leaf extract.

Mortality rates of adult head lice with a ratio of 2:1 was 95%, while the louse eggs only reached 90%. This was because the louse eggs still have a protective membrane and operculum, making it more resistant to mechanical disturbances (physically and chemically). Adult head lice (imago) does not have the protective membrane and has been active in the search for food. So the effect of the plant-pesticide impacts more to the adult head lice.

This study shows that the combination of lime leaf extract and eucalyptus oil can kill *Pediculus humanus capitis* (eggs and adults). Based on this, it is concluded that the best role of plant-based insecticide compound is limonoids (in the lime leaves extract). Therefore, further study is needed to test which concentration of each solution more kill head lice (lethal concentration), associated with the time (lethal time).

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Immobilization of *Aspergillus niger* and *Mortierella* sp. Lipase in Zeolite and Alginate for Liquid Waste Degradation

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ABSTRACT

Lipid containing waste is potentially pollutant for environment since it is insoluble. To take over it, lipase is commonly used to degrade it. Many study had already done to increase lipase activity, one is immobilization study since immobilized enzyme could be used many times depend on its stability. This study is aimed to find out lipase activity produced by *Aspergillusniger*LM 1002 and *Mortierella* sp. LM 1021 which is immobilized in zeolite and alginate. Enzyme activity is measured based on released oleic acid with spectrophotometri λ 715 nm, while lipid degradation is measured based on released oleic acid before and after 24 hour incubation. Both are followed standard methods. Result indicated that activity of lipase immobilized in zeolite is more stable that it in alginate. Lipase produced by consortia in zeolite reaches activity about 29.714 U/ml and lipid degradation about 1.7%.

Keywords : Alginate, zeolite, lipase immobilization, organic waste

INTRODUCTION

Indonesia has potential in the development of production of lipase through local microorganisms diversity (Parenrengi et al., 2011). The use of microbes as producers of enzymes has several advantages, including production costs are relatively inexpensive, can be produced in a short time, have high growth rates and is easily controlled (Fogarty & Weshoff, 1983). The lipase production are being developed in research and application is the production of lipase derived from fungi kingdom (Viruthagiri, 2007). Some types of fungi known have potential to produce lipase enzyme include Aspergillus, Mucor, Rhizopus, and Penicillium. (Sharma et al., 2001).

Lipase (triacylglycerol acyl hydrolases EC 3.1.1.3) is an enzyme hydrolysis form carboxylic ester chain (Castro, 2011) which has a physiological role to hydrolyze triglycerides to diglycerides, monoglycerides, fatty acids, and glycerol (Leblanc, 2004). Lipase is used in a wide range of products and processes in the food industry sector, detergent, pharmaceutical, textile and paper industry (Viruthagiri, 2007). Besides used in the production needs of an industry, lipase is also used as the biodegradation of waste containing lipids generated from industrial processes (Mulyadi, 2013). Lipid is waste that has a significant impact on the environment due to the nature of the fat that is generally insoluble in water (Tresna, 1991).

Price of lipase commercial usually very high because the production process is difficult and can not be reused because it dissolved in the reaction medium (Kirk et al., 2002), so it's important to do a research about techniques reuse of lipase, one of the technique of enzyme immobilization is trapping the enzyme (Datta, 2013) with the aid of the carrier can withstand the enzyme so that the enzyme is expected to be kept within a certain period and the application fee can be reduced (Bornscheuer, 2005). Enzyme immobilization technique can improve the performance of enzymes in organic solvents such as pH tolerance, thermal stability or stability of functional (Wojcieszyńska et al., 2014).

Medium support immobilisation of enzymes can be derived from organic and inorganic materials. This study will be compared between the immobilization of enzymes with alginate and zeolite. Alginate is organic

material which is a polysaccharide produced by brown algae, alginate in the form of calcium alginate has the potential to be able to trap the enzyme (Datta, 2013), while the zeolite is an inorganic materials in the form of a mineral consisting of crystalline aminosilicate and a porous structure that has good ability of adsorption to be able forimmobilizing enzyme (Septiani, 2011).

In Kurniawati study (2014), it was found that the consortium of species of mould has the greatest lipase activity is consortium of *Aspergillus niger* consortium LM 1002 and *Mortierella* sp. LM 1021 with lipase activity of 0.1218 U / mL and value of lipid degradation activity amounted to 62.2507% on the medium used cooking oil waste, so in this study used consortium isolates of A. *niger* LM 1002 and *Mortierella* sp LM 1021 and each of single isolates of the consortium of fungi.

Based on these data, it's important for developingthe optimum production of lipase from the fungus consortium combination in a medium cooking oil waste production and immobilization techniques to determine immobilized lipase activity within a specific time as well as lipid degradation activity of lipase immobilized in organic waste containing lipids.

METHODS

Growth Curve

The basal medium (30 peptone; 2.0 KH2PO4; 0.5 MgSO4.7H20; 0.5 KCl g / L)and 1% cooking oil composition: glucose (3: 1) was inoculated with 10% single or concorsium inoculums. Growth was controlled every 24 hours for 7 days by measuring the value of biomassand presented in the growth curve.

Starter Inoculum

The spores suspension consortia and spores ofsingle isolates of *Aspergillus niger* and *Mortierella* sp. respectively 10% ($1x10^6$ spores / mL) was inoculated in 30 mLbasal medium. Sterilization with autoclave for 15 min , 121^oC , 1.5 atm (Akhtar et.al, 1980). Incubated in a shaker incubator 120 rpm with an incubation period in accordance with the result of the growth curve at room temperature .

Lipase Production

The production medium of lipase were10% starter, 10% tween and basal medium with total volume of 300 mL, and then homogenized to form an emulsion and measured the degree of acidity (pH). Sterilization with autoclave for 15 min, 121^oC, 1.5 atm. Inoculated starter into production medium and incubated for 7 days at40 ° C (Maia *et.al*, 2001).

Enzyme Extraction

The culture was filtered to separate the mold biomass with the supernatant. Then put into a centrifuge bottle each of 10 mL. The next step, centrifuged at 8000 rpm for 15 min, and take the supernatant which is assumed as a crude enzyme and enzyme activity tested.

Lipase Immobilization

Crude enzyme extract that has been obtained is taken as 1 mL of the enzyme, then added 0.3 gram of activated natural zeolite. The mixture was allowed to stand for 1 hour with occasional stirring. Then the mixture was centrifuged for 5 minutes at 1000 rpm (Septiani et.al, 2011). Immobilized lipase activity were further tested at early time, then stored in 4 ° C (Parthu, 2012).

Making alginate bead is done by mixing alginate with a crude enzyme mixture to form a final concentration of 2% (w / v). A mixture of enzymes and alginate extracted and dropped into $CaCl_2$liquid (5% w/v) and allowed to stand for 1 hour at 4 ° C. Alginate beads whichformed are then filtered and rinsed with distilled water (Aqua bidest), then dried in the open air for 1 hour prior to the test process is done (Demirkan, 2011). Then stored at 4 ° C until the time of specified reuse. (Azhar et.al, 2009).

Lipase Activity

Lipase activity tests is done before the test lipid degradation in waste cooking oil. This test is done in a week of the test once every 24 hours. The steps are taken 1 mL of lipase immobilized and put in a different reaction tubes, then add 1 mL of phosphate buffer pH 7 and 1.5 mL of olive oil. This mixture is then incubated on a shaker incubator at room temperature for 30 minutes. After the incubation process is complete, immobilized lipase filtered and washed and then used in the process of lipid degradation test.

A mixture of olive oil that had been incubated added 6N HCl solution of 1 mL and 5 mL of reagent ethanol 96%. The mixture was shaken further strong and the top layer is taken as much as 2 mL, 0.5 mL reagent is then added copper (II) acetate and shaken in the vortex for 1 minute. The absorbance was measured at a wavelength of 715 nm. The concentration of oleic acid formed as a result of the degradation of the lipase can be determined from the measured absorbance values. Oleic acid concentration values are then used to determine the value of lipase activity. Values subsequently lipase activity was calculated by the formula:

$$\text{Enzyme Activity} = \frac{\textit{Oleic Acid Concentration}\left(\frac{\mu mol}{ml}\right)}{\textit{Incubation Time (minute)}} \; x \; \frac{\textit{Total Volume (mL)}}{\textit{Enzyme Volume (mL)}}$$

Determination of Lipid Degradation

The organic liquid waste which tested are cooking oil waste. Waste filtered to separate the impurities. The degree of acidity (pH) in the waste cooking oil measured. Testing was conducted over three days with the test once every 24 hours. The first step taken to test the value of lipid degradation is cooking oil waste and 1.5 mL of immobilized crude lipase addedto calculate its value. This mixture is then incubated on a rotary shaker at 120 rpm for 1 day. After the testing of lipid waste degradation process is complete, immobilized of crude lipase filtered and then washed. After the washing process, immobilized crude lipase stored at 4 ° C for use in the test process in the next 24 hours and at subsequent testing. Measurements were conducted by adding 2 mL sample of liquid waste that has been incubated with 5 mL 96% ethanol. The mixture was shaken further strong and the top layer is taken as 2mL, then added 0.5 mL reagent of copper (II) acetate. The absorbance of the mixture was measured with a spectrophotometer at a wavelength of 715 nm. Results absorbance calculated its concentration with mathematical equations from of oleic acid standard curve, so that would be obtained fatty acid (oleic acid) contained in the sample liquid waste. Degradation value can be determined by the formula:

$$\textit{Degradation Value} = \frac{(\textit{Final Concentration} - \textit{Initial Concentration})}{\textit{Initial Concentration}} \; x\; 100\%$$

RESULTS AND DISCUSSION

Aspergillus niger experiencing log phase on the first day until the 3rd day and then decreased the amount of biomass to the 7th day, so the age of the starter obtained is the 36th hour. In species *Mortierella* sp. and consortium of the species it is known that there is a growth in the log phase on the first day to the 5th day and the decreasing of the number of biomass when entering the 6th and 7th day, so we get theage of the starter at the 60th hour.

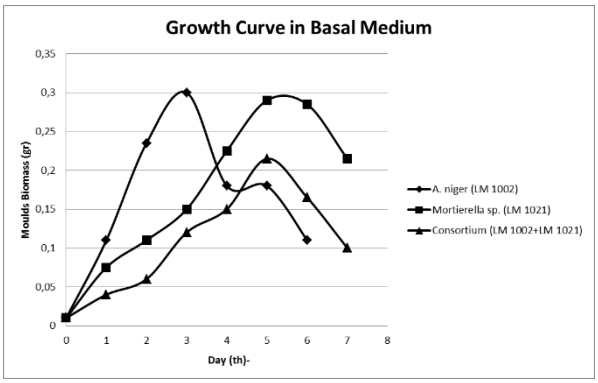

Figure 1. Growth curve of moulds in basal medium.

Test activity is a confirmatory test which show the ability of the enzyme. One unit is defined as the amount of enzyme that catalyses the transformation of one micromole of substrate per minute(Meyers, 1995).

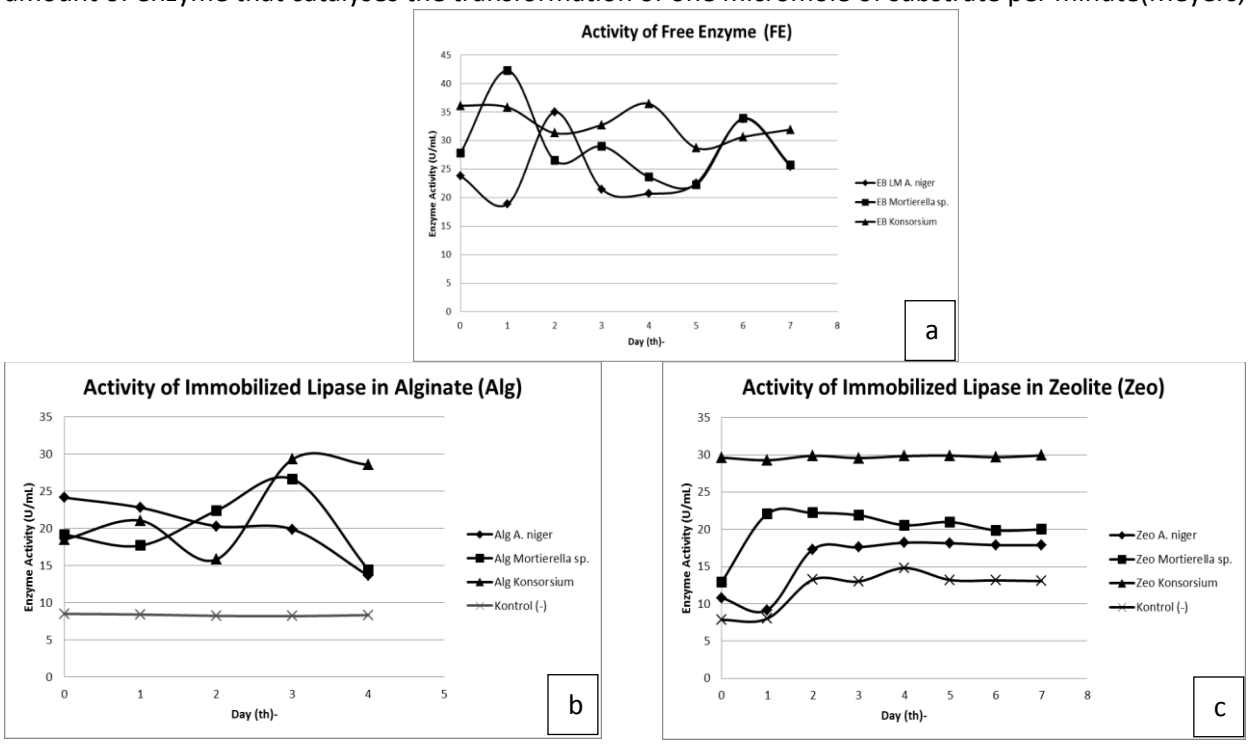

Figure 2. Enzyme activity by free enzyme (a), immobilized lipase in alginate (b), immobilized lipase in zeolite (c).

The most stable free enzyme activity in the lipase produced by the consortium, with an average lipase activity rate of 32.974 U / mL for 7 days (fig 2.a). The highest free enzyme activity with a value of 36.447 U / mL occurred on the fourth day.While in single isolates, the highest free enzyme activity produced by *Mortierella* sp. with an average value of lipase activity for 7 days was 28.888 U / mL and the value of the free enzyme activity was highest in the first day with the value of the activity of 42.262 U / mL. Free enzyme of A. *niger* has an average value of enzyme activity for 7 days, 25.202 U / mL and enzyme activity is highest on 2nd with a value of 34.983 U / mL. The highest value of the lipase enzyme activity produced by *Mortierella* sp. This is consistent with previous research conducted by Pramitasari 2012. In the study mentioned that the mold types *Mortierella* sp. has the highest value of lipase activity.

Free enzyme activity fluctuated significantly, the enzyme activity is not stable for 7 days of testing. The instability of the enzyme activity is influenced by environmental factors such as temperature, pH, and organic solvents. Environmental factors that change may cause the enzymes become denatured and inactivated causing the lower the stability and value of their activities (Lason, 2010), in the presence of environmental factors

affecting fluctuations in the activity of the free enzyme, in this study the activity of the free enzyme acts as control of immobilized enzyme activity in alginate and zeolite.

The highest lipase activities of immobilized crude enzyme in alginate produced by a consortium of *Mortierella* sp. and A. *niger* was 29.295 U / mL on3rd day (Fig.2.b).In single isolate *Mortierella* sp., the highest enzyme activity was present on the third day with a value of 26.647 U / mL and continued to decline in subsequent use. In A. niger species, the highest activity value of 24.148 U / mL occurred on the first day.

The highest value of the lipase enzyme activity produced by a consortium of two isolates. The enzyme activity increased in the third day and then decreased enzyme activity in the next use, while at the A. *niger* enzyme activity has been declining since the 2nd day. The decrease in alginate immobilized crude lipase activity is in line with the study by Anwar et al. 2009. The study states that in every cycle of immobilized enzyme utilization calcium alginate decreases the activity of enzyme. The decrease in enzyme activity is due to enzyme leakage or enzyme diffusion out of the calcium alginate gel beads caused by gel leaching at each end of use. Factors of calcium alginate gel damage also allow for reduced enzyme activity.

The enzyme activity of immobilized lipase crude inzeolites (Fig.2.c) have a fairly good stability when compared to the activity of the free enzyme and immobilized enzyme activity in alginate. Zeolite which has activated has been free from substances of impurities and moisture, so the level of the higher porosity and surface area is also increasing. Along with the increased surface area and porosity of the zeolite, the absorption process can take place in an optimal enzyme. In addition, the immobilization of enzymes by adsorption mechanism has the advantage of allowing it to maintain and increase the activity of enzymes for the immobilization process done by the absorption method, without any modification to other chemical compounds (Elnashar, 2011). Parent (2006) also reported that in addition to having adsorption capability is quite good with good stability, zeolites have the ability as a catalytic agent of reaction in the presence of ion exchange process and has been widely applied in petroleum processing and detergent industries, so that the zeolite is possible to have a role in the catalyst degradation of lipids into fatty acids and glycerol with a fairly good stability.

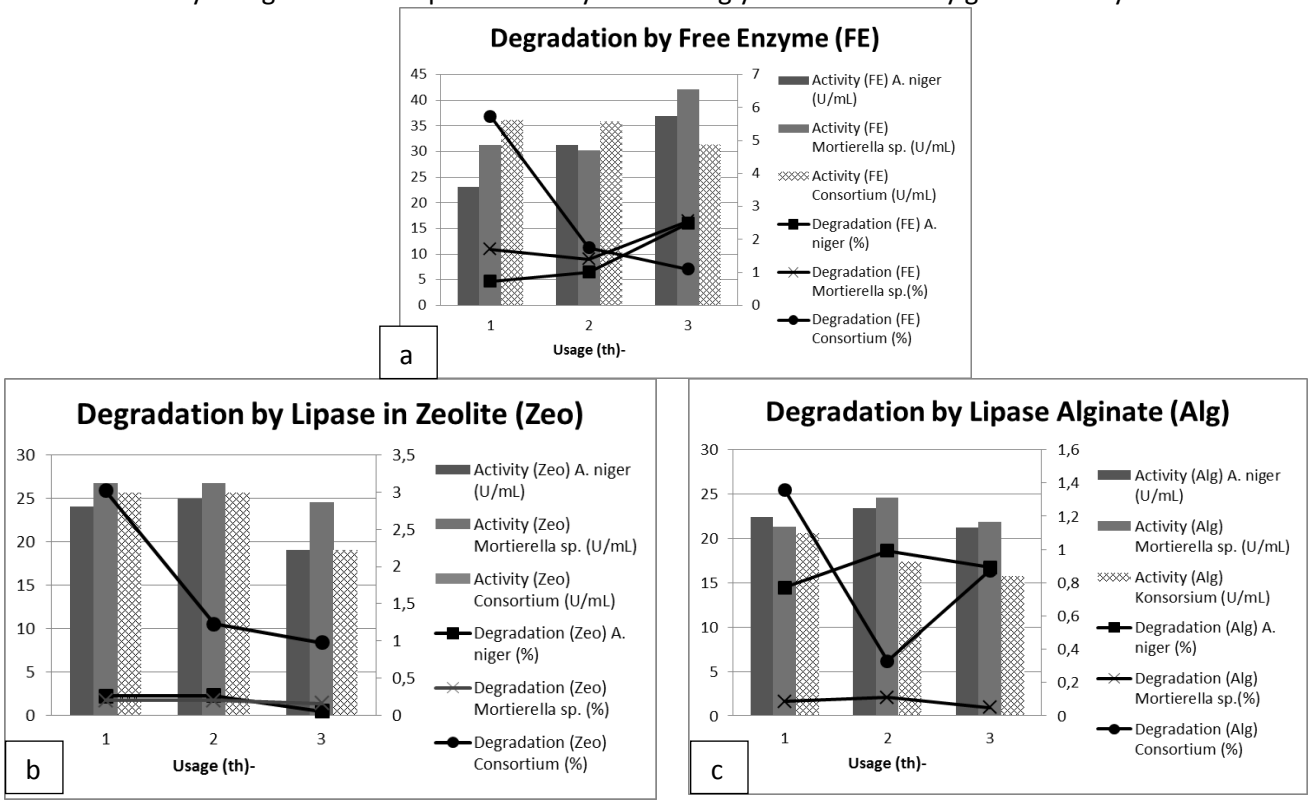

Figure 3. Degradation by free enzyme (a), degradation by immobilized lipase in zeolite (b), degradation by immobilized lipase in alginate (c).

Enzyme activity and degradation value increased up to the third day, this is possible due to the change of free enzyme condition during storage process (Fig.3.a). Turbidity that occurs in the enzyme can affect the value of absorbance during activity testing and degradation test takes place, the higher the turbidity then the absorbance value can also be higher so that the test value of enzyme activity and lipid degradation increase. Visualization of crude enzyme opacities is shown in the figure 4.

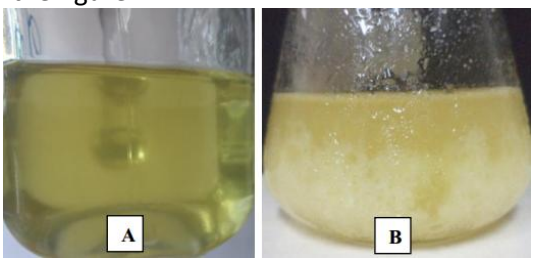

Figure 4. The turbidity ofA. *niger crude* lipase (A: The enzyme storage in the first day. B: The enzyme storage > 7 days).

The value of free enzyme activity from *Mortierella* sp. Fluctuates from first to third use. Enzyme activity decreased in the second use, from 31,224 U / mL to 30,286 U / mL and then again increased in third usage to 42,105 U / mL.The degradation value was directly proportional to enzyme activity, fluctuation of degradation value also occurred in accordance with enzyme activity, on the second day use degradation decreased from 1.702% on first day use to 1.398% and then increased to 2.556%.

Stability activities and capabilities in line with the results of crude lipase activity of *Mortierella* sp. which has been tested previously, while the use of the free enzyme produced by a consortium of two moulds, the first day of usage in the third day of enzyme activity decreased, the enzyme activity decreased in the usage in the second day, from 36.139 U / mL in the usage in the the first day be 35.839 U / mL and then declined in the usage of the third day be 31.321 U / mL. The value of the degradation is directly proportional to the activity of the enzyme, impairment degradation also occurs in accordance with the enzyme activity, the use of the second day of degradation decreased of 5.733% in the use of the first day be 1.743% and then continued to decline in the use of the third day be 1.098%. Decreased activity of the enzyme or enzyme instability and capability degradation that can occur due to the influence of the environment such as temperature and pH potentially denaturated the free enzyme (Ogonowski, 2010).

The lipase from A. *niger* which immobilized in zeolite, on the first day to the second day of use increased enzyme activity, 24.028 U / mL on the first day of use becomes 24.936 U / mL on the 2nd day. The increase in the value of the enzyme activity does not occur significantly because it is still within the range of values so that the same can be said that the value of the enzyme activity is stable. On the third day of use activity of the enzyme decreased be 19.023 U / mL. The value of the degradation is directly proportional to the activity of the enzyme, the first and second day of use, it has value 0.254% on the first day of use be 0.264% and then decreased in the use of the third day be 0.055%. Values activity of the enzyme is still in a good range and prove that the first and second day, the catalytic reaction of lipase or zeolite run well, but on the third day of a process had impairment in activity and degradation value.

On the use of immobilized crude lipase of *Mortierella* sp. in zeolite, The first day to the second day of the enzyme activity increases, 26.752 U / mL in the use of the first day be 26.714 U/mL on the 2nd day, the increase in the value of enzyme activity at the first and the second days do not happen significantly because it is still within the range of values so that the same can be said that the value of the enzyme activity is stable. On the third day of activity of the enzyme decreased be 24.583 U/mL. The value of the degradation is directly proportional to the activity of the enzyme, the first and second day of use decreased, 0.200% on the first day of use be 0.198% and then decreased in the use of the third day be 0.159%. Values activity of the enzyme is still in a good range and prove in the first and second day, the catalytic reaction of lipase or zeolite run well, but on the third day of a process had impairment in activity and degradation value.

On the use of immobilized lipase from a consortium of two molds in zeolite, first day to use to the third day of enzyme activity is fluctuating, ie 25.648 U / mL in the use of the first day be 25.701 U / mL on the third day,

the increase in the value of enzyme activity in the usage of the first day and the second day does not happen significant because it is still within the range of values so that the same can be said that the value of the enzyme activity is stable. On the third day of use continue activity of the enzyme decreased be 19.128 U / mL. Value degradation degradation in the first day has a value of 3.020% use decreased, ie 1.227% on the third day of use continue degradation decreased be 0,974%. These results are consistent with the results of tests performed on both single isolates, value the activity of the enzyme is still in a good range and prove in the first and second day, the catalytic reaction of lipase or zeolite run well, but on the third day of a process had impairment in activity and degradation value.

Impairment of degradation can occur is made possible because the bonding in the process of adsorption of enzymes by the zeolites occurs reversibly and interaction between the enzyme with the zeolite is not too strong, namely the interaction of van der waal so between crude enzyme with absorbent easily detached (Septiani, 2011) and the value of the activity generated by the catalytic reaction of A. *niger* lipase which immobilized in zeolite and not produced by the enzyme so that the degradation value close to 0 or no degradation process occurs. Another factor that would allow non-occurrence of degradation processes on the third day was the condition of used cooking oil waste which is not homogeneous and possible have a lot of fatty acids resulting from the hydrolysis process during the frying process at high temperature (Mardina, 2012), the water vapor produced during the frying process and causing hydrolysis of the triglycerides, free fatty acid (Kulkarni, 2006), so lipase or zeolite does not do much lipid-solving process.

Based on the figure 8, the use of lipase from A. *niger*which immobilized in alginate in the first day to the third day of use, enzyme activity is fluctuating, from 22.429 U / mL on the first day of use becomes 23.442 U / mL on second day. On the third day of use continue activity of the enzyme decreased be 21.221 U / mL. The value of the degradation is directly proportional to the enzyme activity due to a fluctuation, on the first day of use has a value of 0.772% degradation of the second usage has increased the value of degradation that is 0.993% on the third day of use, degradation values decreased becomes 0.891%.

On the use of lipase from *Mortierella* sp. which immobilized in alginate, on the first day to the second day of use increased enzyme activity, from 21.274 U / mL on the first day of use becomes 24.605 U / mL on second day. On the third day of use, activity of the enzyme value decreased be 21.836 U / mL. The value of the degradation is directly proportional to the activity of the enzyme, the first and second day values are 0.0849% on the first day of use be 0.111% and then decreased in the use of the third day be 0.0498%.

On the use of lipase from the consortium isolateswhich immobilizes in alginate, on the first day until the third day usage decreased enzyme activity, The values are 20.651 U / mL on the first day of use becomes 17.412 U / mL on day two. On the third day of use, activity of the enzyme value decreased be 15.833 U / mL. The value of the degradation does not directly proportional to the enzyme activity due to a fluctuation, on the first day of use has a value of 1.359% degradation of the use and decreased be 0.486% and on the third day of use has increased the value of degradation becomes 0.874%.

The decline and fluctuations in the value and the degradation activity is in line with research from Anwar *et.al* (2009) which states that the use of immobilized enzymes in alginate will decline in the use of the three times caused the leakage of enzyme during the washing process takes place.

The highest degradation value generated by the free enzyme lipase of the consortium with a value of 2.858%. For immobilized lipase, the highest degradation value generated by immobilized lipase of isolates consortium with a value of 1.740%. The average value of the highest degradation isolates produced by the consortium, it is caused by the degradation of lipid metabolic activity complementary (Anggraeni, 2014) among A. *niger* and *Mortierella* sp.

CONCLUSION

The activity of immobilized lipase in zeolite more stable than in alginat during storage the 7^{th} days. Theimmobilized lipase can degrade over two times of use. the highest enzyme activity produced by a consortium

of two isolates with a value of 32.974 U / mL for the free enzyme and the highest average value of the activity produced by immobilized lipase inzeolite with a value of 29.714 U / mL. The average value of the highest lipid degradation produced by a consortium of free lipase and immobilized lipase in zeolite. Thus, the best immobilization support medium in this study are zeolites.

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Improving Properties of Sweet Potato Composite Flour: Influence of Lactic Fermentation

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ABSTRACT

The use of locally grown crops such as sweet potato as raw material for composite flour is considered advantageous as it reduces the importation of wheat flour. However the use of native sweetpotato flour has drawback properties when applied in the food. This study was aimed to modify sweet potato flour through six methods of lactic fermentation (spontaneous, pickle brine, *Lb plantarum, Lc mesentereoides, a mixed of Lb plantarum and Lc mesentereoides,* and *mixed of Lb plantarum, Lc mesentereoides* and yeast) to increase its properties in composite flour. Composite flours were obtained after fermentation of sweet potato slices for 48h in the proportion of 50% sweet potatoes flour and 50% wheat flour. pH, moisture content, swelling power, solubility, and pasting properties were determined for the fermented and unfermented composite flours. The results indicated that the composite fermented flours had better properties than those of non fermented flour. Fermentation increased swelling power, moisture content, meanwhile, solubility, and pH, deacresed. Amylose leaching, however, was not significantly affected by the fermentation process.

Keywords: Composite sweetpotato flour, lactic acid fermentation.

INTRODUCTION

Composite flour is considered advantageous in developing countries as it reduces the importation of wheat flour and encourages the use of locally grown crops . One of the potential locally raw materials used in composite for substitute the wheat is white sweet potato (SP). This crop can be processed into white sweet potato flour and then it could be used as a substitute for wheat flour (20 %-80 %) in baked goods, cakes and noodles. However, the use of native sweetpotaoes for composite flour still has drawback properties. The uses of this root in substitution of composite flour in which the product still acceptable for consumers is generally only up to 20 % in the manufacture of vermicelli (Collado *et al*., 2001 and Lase *et al*., 2013) and noodles (Lee *et al*., 2005 and Chen *et al*., 2006). In addition, the color of SP noodles is darker and less bright, and its texture is low elastic (Sugiyono *et al*., 2011; Chen, 2006). Addition of sweet potato flour in vermicelli making is less consumer preferences for color products (Rizal, 2012). Thus, modification of white sweet potato flour to improve its physical and sensory properties is need to pay attention.

Physical and sensory properties of white sweet potato flour can be improved in many ways such as chemistry, physics, and microbiology methods. Chemically modifying sweet potato flour could be done with the addition of sodium tri polyphosphat during the process of making dough (Retnaningtyas and Putri, 2014),or with carboxyl metyl cellulosa addition (Mulyadi *et al*., 2014). In physically method, modification could be done trough high-moisture treatment (Kusnandar, 2009; Lase *et al*., 2013); while in microbiologically method by using of either fermentation (Yuliana *et al*., 2014; Pratiwi, 2014; Dewi, 2014) or enzyme application. Chemically modifying sweet potatoes flour is relatively easy to do, however, the use of chemical additives is feared to affect the human health.

In physiccally, the product is relatively safe to consume but it difficults in the use of high temperature and humidity setting, especially if the device is not adequate. While eznyme application is relatively expensive. In this research, fermentation was choosen to improve the physical and sensory properties of sweet potato as it was relatively easy and safety.

Fermentation as a meant to improve propeorties could be done either with or without addition of culture. Some of the examples were fermentation of casava by using *Lactobacillus plantarum* and *Saccharomyces cerevisiae* culture to improve their properties in "mocaf" (Mutia, 2011), fermentation of sweet potatoes pikel with either *Leuconostoc mesenteroides* (Yuliana *et al*., 2013) or a mixed cultures of *Lactobacillus plantarum* and *Leuconostoc mesenteroides* (Yuliana *et al.*, 2013), and with addition of *Lactobacillus plantarum* (Yuliana *et al*., 2014), as well as fermentation of sweet potato in spontaneously method (Pratiwi, 2014). In this study fermentation was carried out by using several cultures to improve properties of sweet potatoes. The best composite flour made of fermented sweet potatoes was then determined.

MATERIALS AND METHODS

Materials

The materials used in this study were: white sweet potatoes tubes, variety of ciceh from Sekincau Liwa, purchased in traditional markets in Bandarjaya-Lampung; starter *Lactobacillus plantarum FNCC 0123* and *Leuconostoc mesenteroides FNCC 0023* (Laboratory of Food Universitas Gajah Mada), *Saccharomyces cereviceae* in the form of commercial ragi (Fermipan), wheat flour (brand Chakra, Bogasari), eggs, sugar (Gulaku), salt (Refina) and cooking oil (brand Filma). Chemicals used in these experiments were distilled water, NaCl, NaOH, H2SO4, CaCO3, Iodine solution, 95% ethanol, acetic acid 1 N, and pure amylose.

Preparation of starters

(1) Lactobacillus plantarum *or* Leuconostoc mesenteroides *starter.*

Two ml culture of *Lactobacillus plantarum* or *Leuconostoc mesenteroides* in 10 ml MRS Broth was taken and each was then tranfered in to 18 ml of sterile MRS broth and incubated for 24 hours ,at 37°C. After that, each culture was taken as much as 2.5 ml and transfered into 22.5 mL sterile MRS broth and again incubated for 24 hours at 37°C. To get working starter, each culture was then tranfered into 250 ml of Erlenmeyer contained 215 ml sterile MRS broth and incubated for 24 hours, at 37°C.

(2) Preparation of Saccharomyces cerevisiae *starter.*

One gram of *Saccharomyces cerevisiae* ragi was poured into 100 ml of steril distilled water then was homogenized. *Saccharomyces cerevisiae* culture was then ready for use.

(3) Preparation of pikel brine starter.

The sweet potato cubes weighed as much as 40 g were inserted into 150 ml of bottle fermentation contained 110 ml of saline solution. This mixtures were then pasteurized at 72^0C - 73^0C and then was fermented for 4 days at room temperature. The brine of this fermented cubes was then ready for starter use.

Fermentation of Sweet Potatoes

Sweet potatoes were peeled and washed and then sliced using a slicer size of 1 mm. Sweet potatoes slices were taken as much as 1.8 kg and then were put in a sealed container volume of 6 L. Sugar solution (2.5L) contained 1% sugar and 3% salt was then added. This mixture was innocullated with a starter as much as 5% (v/v)

in accordance with the treatments and then were fermented for 2 days (48 hours) at room temperature. Treatments in these study consisted of: (A) Control without fermentation, (B) Spontaneous fermentation (without starter added), C. Pikel brine starter, D. *Lactobacillus plantarum* starter with a cell density of 10^6 cells /mL, (E) *Leuconostoc mesenteroides* with a cell density of 10^6 cells/mL, (F) a mixed starter of *Lactobacillus plantarum* and *Leuconostoc mesenteroides* with a cell density of 10^6 cells /mL, and (G) a mixed starter of *Lactobacillus plantarum, Leuconostoc mesenteroides,* and *Saccharomyces cerevisiae* with a cell density 10^6 CFU/g.

Production of Sweetpotatoes Flour

The sweetpotatoes slices either fermented or non fermented as a control are washed, drained and dried in an oven blower (British foyer) at tmperature of 60◦ C for 24 hours , until water content of ± 6-8 % was reached. The dried slices were then ground using a grinder (Rulb Fanc) and sieved using a 80 mesh siever. These flours were then packed in plastic lid tightly and kept for further analysis .

Composite Flours

Composite flours were made by mixing of sweet potato flour treatments above with wheat flour in the ratio of 50% : 50% by using a mixer for 5 minutes. These composite flours were then analyzed to determine moisture AOAC (1995), pH , solubility and swelling power (Deng *et al*., 2013), and amylose leaching (Kusnandar *et al*., 2009).

Research methods

The experimental design used was the complete randomized block design (CRBD) in one factorial with four replications. The treatment consisted of 7 types of composite flour made from non fermented sweet potato-wheat flour (A) as a control, and 6 fermented sweetpotatoes-wheat flour (B,C,D,E,F,G,H). Data of modified sweet potato compsite flour were analyzed by using analysis of variance. Duncan test was used to determine differences among treatments at 5% level.

RESULTS AND DISCUSSION

Water Content and pH

Water content and pH of composite flours are presented in Table 1. Results showed that the water content of fermented flour tends to be higher than the control while the pH of fermented composite flour was lower. The increase of water content on fermented flour could be attributed to 48 hours of soaking stage during fermentation process. The water content, neverthelees, was still below of SNI maximum water content of flour (less than 13%). The water content is one of important components in the manufacture of food products because they affect the shelf life of the food product. In addition, water may affect the appearance, texture and flavor of food (Sudarmadji, 1997).

Table 2. Water and pH of sweet potato composite flour.

Treatments	Water Content (%)	pH
Control	4,25±0,30[a]	6,22±0,03[a]
Spontaneous	4,81±0,85[b]	5,35±0,06[b]
Pickel Brine	5,08±1,03[b]	4,76±0,07[c]
Lb	4,52±0,73[ab]	4,31±0,11[d]
Lc	4,70±0,80[b]	5,32±0,20[b]
Lb + Lc	3,99±0,11[a]	4,85±0,09[c]
Lb + Lc + Yeast	4,11±0,18[a]	5,39±0,14[b]

Number followed by different letters in the same column shows the difference at 5% level by Duncan test. Lb = *Lactobacillus plantarum* ; Lc = *Leuconostoc mesenteroides*; Yeast = *Saccharomyces cerevisiae*.

Composite fermented flour had pH values (4.31 to 5.39) that lower than the compopsite control flour (6.22) and wheat flour (6.11). Lactic acid bacteria such as *Lactobacillus plantarum* and *Leuconostoc mesenteroides* may produce amylase that hydrolyzed most of the starch into monosaccharides and others metabolites as a source of energy. These were then converted to organic acid mainly as lactic acid and substantial amount of acetic acid and caused the pH to be drop (Oghonejoboh, 2012). The final pH was affected by microbial cultures used in each sample and among them, the pH of composite flour produced by *Lactobacillus plantarum* was the lowest, amounted to 4.31. *Lactobacillus plantarum* was homofermentatif lactic acid bacteria which has high amylolytic activity, and classified as a strong lactic acid producer (Sharp, 1979; Salminen and Wright, 1993). Mean while, the pH of the flour derived from a mixed culture of *Lactobacillus plantarum, Leuconostoc mesenteroides,* and yeast had value close to neutral, that was 5.39. This was likely due to the lactic acid produced by *Lactobacillus plantarum* and *Leuconostoc mesenteroides* was used by *Saccharomyces cerevisiae* and reformed it into a secondary metabolite such as alcohol.

Sollubility at Different Temperatures and Amylose Leaching.

Table 3 shows that fermentation deacreased sollubility and amylose leaching. The sollubility was observed to deacrease with increase in temperature (70-95C) with the composite fermented flours exhibiting significant amylose leaching when compared with the composite control SP flour, except for the pickle brine treatment. Lower sollubility in fermented flour than the control was probably due some of the starch to have been degraded into shorter polymer chains as action of enzyme produced by lactic acid bacteria. These shorter polymers e.i simple sugar were more soluble and probablly were dissolved in fermentation medium resulted in longer chain polymer retain in the flour.

Tabel 3. Solubility and amylose leaching.

Treatments	*Sollubility* 70°C (%)	*Sollubility* 85°C (%)	*Sollubility* 95°C (%)	*Amylose Leaching* (%)
Control	9,63 ± 0,36^{c}	13,01±0,50^{a}	10,52±0,35^{ab}	0,019±0,003^{b}
Spontaneous	7,35 ± 1,39^{ab}	10,39±1,26^{b}	11,42±0,45^{a}	0,014±0,002^{bc}
Pickel Brine	6,31 ± 0,20^{a}	9,65±0,67^{c}	10,85±0,86^{ab}	0,080±0,09^{a}
Lb	7,30 ± 0,91^{ab}	9,66±0,76^{c}	11,19±0,77^{ab}	0,015±0,004^{abc}
Lc	7,23 ± 0,54^{ab}	8,70±0,59^{d}	9,67±0,52^{b}	0,042±0,001^{ab}
Lb + Lc	7,13 ± 0,76^{ab}	10,16±0,64^{b}	10,11±1,19^{ab}	0,026±0,004^{abc}
Lb + Lc + Yeast	6,75 ± 0,45^{a}	9,56±0,58^{c}	10,53±1,02^{ab}	0,028±0,003^{ab}

Number followed by different letters in the same column shows the difference at 5% level by Duncan test. Lb = *Lactobacillus plantarum* ; Lc = *Leuconostoc mesenteroides*; Yeast = *Saccharomyces cerevisiae.*

Among the fermented flours, the sollubility of fermented flour treated by mixed culture of *Lactobacillus plantarum*, *Leuconostoc mesenteroides*, and *Saccharomyces cerevisiae* showed the lowest and stable. According to Collado *et al*. (2001), flour with low solubility and stable values is best flour for raw material noodles use. Starch structural such as chain length distribution of amylose and amylopectin might caused differences of starch solubility among samples. Bello-Perez *et al*. (2000) reported that the distributions of chain length in the starches cause differences in Solubility. According Fleche (1985), when the starch molecule was completely hydrated, these molecules began to spread to the media on the outside. Molecules that first came out were molecules that had shorter chain e.i amylose. The higher the temperature the more the starch molecules will out of the starch granules.

Table 3 shows that amylose leaching of fermented and control was comporable low, except for pickle brine treatment. Amylose leaching (release amylose) was the process of release of amylose during the gelatinization process. High amylose on the surface of the noodles after cooking can increase the level of stickiness as reported in corn moodles (Kusnandar, 2009). The low amount of amylose off during the heating due to the large number of well-amylose forms complexes with amylose, amylopectin, or fat. Complex bond formation caused the starch had a bond that was compact and tight, so that the amount of amylose off lower (Gunaratne and Hoover, 2001).

Swelling Power.

The swelling power of composite SP flour at different temperatures is shown in Table 4. Generally, the swelling power was observed to increase with increase in temperature (70 to 95◦C) with the composite fermented samples exhibiting significant ability to swell when compared with the composite control flour. Nevertheles, there was no significant different value among the fermentation treatments. Increase in swelling power of flour as a result of fermentation treatment is in conformity with earlier reports for fermented white sweet potatoes (Yuliana, 2014) and fermented moringa flour (Oleyede *et al*., 2016).

Table 4. Swelling power of compposite SP flour.

Treatments	Swelling Power 70°C (%)	Swelling Power 85°C (%)	Swelling Power 95°C (%)
Control	10,46±0,05[a]	9,36±0,32[c]	10,82±0,46[c]
Spontaneous	9,82±0,11[b]	10,90±0,75[b]	12,84±0,42[a]
Pickel Brine	9,31±0,36[c]	11,93±0,77[ab]	12,80±0,27[a]
Lb	9,41±0,39[bc]	11,16±0,58[ab]	12,03±0,64[b]
Lc	9,52±0,45[bc]	11,78±0,46[ab]	12,28±0,84[ab]
Lb + Lc	9,53±0,35[bc]	10,87±0,55[b]	12,23±0,15[ab]
Lb + Lc + Yeast	10,22±0,41[a]	12,20±0,25[a]	12,87±0,32[a]

Number followed by different letters in the same column shows the difference at 5% level by Duncan test. Lb = *Lactobacillus plantarum* ; Lc = *Leuconostoc mesenteroides*; Yeast = *Saccharomyces cerevisiae.*

Hydrolysis of starch granules during fermentation, leading to a lesser structural rigidity in comparison to fermented sweet potato flour. Shorter starch chains as result of these hydrolysis process then tend to be easy absorbed water. Claver *et al*., (2010) reported that when temperature increase and vigorous starch break intermolecular bonds, allowing hydrogen bonding sites to accommodate more water molecules. Water absorbed of each starch granule would make the starch granules swell and increase the swelling power (Odedeji and Adeleke, 2010).

CONCLUSIONS

Fermentation showed beneficial effects on the physicochemical and pasting properties of SP flour. Fermentation significantly increased the swelling power, moisture content, meanwhile, solubility, and pH deacresed. Amylose leaching, however, was not significantly affected by the fermentation process. The best fermentation treatment in this study was either a mixed culture of *Lactobacillus plantarum , Leuconostoc mesenteroides* and y*east* or mixed culture of *Lactobacillus plantarum* and *Leuconostoc mesenteroides.*

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Productivity of *Spirulina* sp. in Modified Karst Water Medium

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ABSTRACT

Spirulina sp. potentially lives in karst water because it contains minerals that support this organism. The aim of this research was to analyze the productivity of *Spirulina* sp. grown in karst water medium with different doses of nutrients supplementary. This research had five treatments with three replications during 18 days observation. Dry biomass was obtained from the conversion of cell density that measured using spectrophotometer at 680 nm. Water quality parameters that measured were temperature, light intensity, salinity, pH, and nutrients. Those parameters were ranged normally and stable during observation, except the pH that quite vary among all treatments. Ammonium, nitrate, and orthophosphate decreased, but nitrite increased. *Spirulina* sp. was able to grow in all treatments, entered the exponential phase at first day, and had high productivity along first six days observation. The productivity of *Spirulina* sp. that grown in purely karst water medium was as high as in nutrient modified karst water medium.

Keywords: Dry biomass, karst water, nutrient, productivity, *Spirulina* sp.

INTRODUCTION

Plankton is free living aquatic microorganism that consists of phytoplankton (autotrophic plankton) and zooplankton (heterotrophic plankton). This community has important ecological roles in aquatic ecosystem, especially as energy source for higher trophic levels. It means that plankton has nutritive value or any essential substances. Phytoplankton is composed by many kinds of microalgae.

Microalgae are a growing source of human nutritional products and could become a future source of sustainable commodities, from foods and feeds, to, possibly, fuels and fertilizers (Ali and Saleh, 2012). *Spirulina* sp. is one of microalgae that proved as beneficial microalga with high content of protein, amino acid, tocopherol, vitamins, and minerals (Vonshak, 1997 in Choi *et al.,* 2008; Richmond, 1980 in Abd El-Baky *et al*., 2003). It means that *Spirulina* sp. has a high market value (Santilan 1982 and Cohen 1995 in Abd El-Baky *et al*., 2003). Recently, *Spirulina* sp. was explored as material for medical and biological research, and essential substance source for cosmetics.

The high demand of *Spirulina* sp. leads to develop simple and economics medium instead of the artificial formulated medium. One of potential medium for *Spirulina* sp. is karst water that contains many kinds of mineral, such as Ca, Mg, C, O, Fe, Mn, Zn, F, Cl, and many cations and anions (Surono, 2009; Gushilman, 2010). Those minerals support the growth of microalga.

The productivity of *Spirulina* sp. is an indicator of suitable medium for the growth of the microalga. In order to understand the potential of karst water as microalgal growth medium, the aim of this research was to analyze the productivity of *Spirulina* sp. grown in karst water medium with different doses of nutrients supplementary.

LITERATURE REVIEW

Karst

The term of karst was originally came from 'Krast', an area at the border of Dinaric, previously Yugoslavia (Croatia, Bosnia-Herzegovina, Slovenia) with north part of Italy (Soenarto, 2006). Those areas were covered by limestone rocks.

Karst area is spread around the world. In Indonesia, it covers about 15.4 million hectare at almost all part of the country. One of them is in West Java. Bogor District is one part of West Java with karst that exploited as rock mining area that created sinkholes, especially in Karst Ciampea, Cibinong, Ciseeng, and Cileungsi (Yoga, 2011).

Potential of Karst Water

Karst sinkhole waters has rich soluble mineral of sediment. The main chemical elements are calcium, magnesium, carbon, and oxygen; the cations are Fe, Mn, and Zn; the anions are CO_3^{2-}, SO_4^{2-}, OH, F^-, and Cl; then, the trace minerals are B, Ba, P, Mg, Ni, Cu, Fe, Zn, Mn, V, Na,U, Sr, Pb, and K (Sutriadi *et al*., 2005; Surono, 2009; Gushilman, 2010). Otherwise, as karst is a good water storage, considerable organic material, either as waste from anthropogenic activities, debris, or feces, are stored inside (Maulana, 2011). Those minerals composition could be utilized as nutrient component for microalgal growth.

Potential of *Spirulina*

Microalgae are a growing source of human nutritional products and could become a future source of sustainable commodities, from foods and feeds, to, possibly, fuels and fertilizers (Ali and Saleh, 2012). *Spirulina* sp. is one of microalgae that proved as beneficial microalgae with high content of protein, amino acid, tocopherol, vitamins, and minerals (Vonshak, 1997 in Choi *et al.,* 2008; Richmond 1980 in Abd El-Baky *et al*., 2003). It means that *Spirulina* sp. has a high market value (Santilan, 1982 and Cohen, 1995 in Abd El-Baky *et al*., 2003). Recently, *Spirulina* sp. was explored as material for medical and biological research, and essential substance source for cosmetics.

The Determination of Biomass and production of Microalga

There are many approaches to estimate the biomass of *Spirulina* sp. One of them was observed indirectly using optical density (OD) approach (Olaizola and Duerr, 1990). From the biomass, there is a calculation of its productivity (Olaizola and Duerr, 1990) and specific growth rates (Trabelsi *et al.,* 2009 in Chainapong *et al.,* 2012).

MATERIALS AND METHODS

The research was conducted in the Laboratory of Plankton Research, Division of Aquatic Productivity and Environment, Department of Aquatic Resources Management, Faculty of Fisheries and Marine Sciences, IPB. The research was set experimentally.

The research was run in two steps, the preliminary and the main experiments. The proper of the preliminary experiment was to learn the ability of *Spirulina* sp. to live in two kinds of karst water, the Ciampea and the Ciseeng karst water.

The stock of *Spirulina* sp. was acquired from the Laboratory of Aquatic Product Biotechnology, Department of Aquatic Product Technology. This species was characterized as marine microalga that lived in

saline water, and cultured in *Spirulina* Medium (SM), that has saline condition (20‰). The inoculum for each treatment was 38.93 mg dry biomass, one day before the first day observation, and stated as day 0 (D-0).

The two karst waters have significantly difference in physical and chemical characteristics, especially temperature of its spring water and salinity. The Ciampea karst water has normal temperature, as its environment, and the salinity was as similar as the common freshwater has. In contrast, the Ciseeng karst water was measured as warm, with temperature about 40°C, and salt with salinity about 30-35‰. Therefore, for the preliminary experiment, those two karst water were modified to make a certain saline water without any significantly change of its physical and chemical character as karst water.

The result of preliminary experiment showed that *Spirulina* sp. could live and grow in Ciseeng karst water. Furthermore, the Ciseeng karst water was used as medium in the main experiment. The Ciseeng karst water was modified by enhancement of nutrients from SM at several levels, with conditioning of salinity that was set at 20‰.

The main research was designed as simple random in time experiment (Mattjik and Sumertajaya 2006) to analyze the productivity of *Spirulina* sp. in modified Ciseeng karst water. There were five suplementary nutrient levels of treatment with three replications and 18 days of observation. Those were culture medium with purely karst water (P1), karst water 75%-SM 25% (P2), karst water 50%-SM 50% (P3), karst water 25%-SM 75% (P4), and purely SM (P5).

The biomass of *Spirulina* sp. was observed indirectly using optical density (OD) approach, based on Olaizola and Duerr (1990). The formulation in calculating dry biomass from OD value is as follows.

$$X = (0.5273 \times OD_{680}) - 0.0138$$

with:

X = dry biomass (g/L)

OD_{680} = *optical density* value

The productivity of *Spirulina* sp. was calculated based on the formulation that delivered by Olaizola and Duerr (1990) as follows.

$$P = \frac{X_i - X_0}{t_i}$$

with:

P = productivity (g/L/hari)

X_0 = early biomass at t_0 (g/L)

X_i = last biomass at t_i (g/L)

t_i = time intervals from X_0 to X_i (days)

The specific growth rate was calculated based on Trabelsi *et al.* (2009) in Chainapong *et al.* (2012) as follows.

$$\mu = \left(\frac{(\ln \bar{X}_{expi} - \ln \bar{X}_{exp0})}{t} \right) \times 100$$

with:

μ = specific growth rate (/day)

$\bar{X}_{expi}$ = biomass of last day of exponential phase (g/L)

$\bar{X}_{exp0}$ = of first day of exponential phase (g/L)

t = time intervals from $\bar{X}_{exp-0}$ to $\bar{X}_{exp-i}$ (days)

The water quality parameters to illustrate the condition of environment were measured along the experiment. The water quality parameters, such as temperature, light intensity, and pH were observed daily. Meanwhile, the nutrients condition was analyze three times at day 0, 9, and 18.

Statistical data analysis was run to understand the difference of response among each treatment and to determine the most efficient modified karst water medium. The significance difference of *Spirulina* sp. biomass and productivity were analized using ANOVA, and continued by Duncan multiple range test analysis (Walpole, 1993; Widiharih, 2001). Furthermore, a matrix approach was used to determine the best modified karst water as substitute alternative medium of *Spirulina* sp.

The determination matrix was based on weight and score decision approach. The total weight value was 20, that comprise of last biomass reached (2), specific growth rate (2), harvest time (4), harvest biomass (4), cost (4), and price (4). Each of parameter was scored into 1-5, matched up to its condition. Hence, the maximum multiplication value of weights and scores was 100.

RESULTS AND DISCUSSIONS

Results

The environment of culture medium of all treatments was controlled as suitable for culture activity. The condition of temperature, light, salinity, and pH were in optimum range for the *Spirulina* sp. (Table 1). The increase of pH value was related to the addition of *Spirulina* medium. Santosa (2010) mentioned that temperature, light, and pH are important factors for the growth of *Spirulina* sp.

Table 1. Environmental condition of all *Spirulina* sp. treatments

Parameters	Unit	P1	P2	P3	P4	P5	Optimum*
Temperature	˚C	35–37	35–37	35–37	35–37	35–37	37
Light	Lux (x100)	23–25	23–25	23–25	23–25	23–25	25
Salinity	‰	20	20	20	20	20	20
pH	-	8.0–8.5	8.0–8.5	9.5–10.0	9.5–10.0	10–10.5	10

* Based on Rafiqul *et al*. (2005) for temperature, light, and pH; Aiba and Ogawa (1977) for salinity

The growth rate of *Spirulina* sp. was calculated from the development of its dry biomass. The microalgal growth patterns of all treatments were shown by Figure 1. The biomass was increase in all treatments and did not showed declination phase at the end of observation, with the highest value at treatment P5 (597.87 mg/L) and the lowest at P1 (477.12 mg/L). The exponential phase was started at the D-1 to D-7. The population growth in this phase is high, up to several times from the early density (Fogg and Thake, 1987 in Setyaningsih *et al.,* 2011).

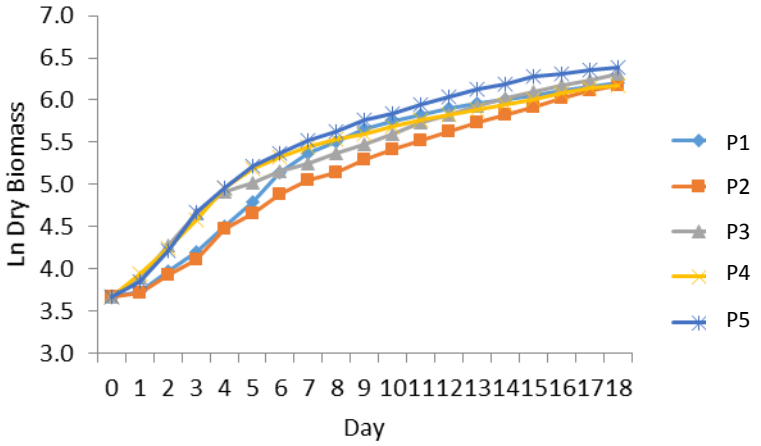

Figure 1. Growth pattern of *Spirulina* sp. of all treatments.

The productivity describes the ability of microalga to produce biomass that calculated daily. Besides, the specific growth rate describes the rate of biomass change in a specific period of time, along the exponential phase periods. The productivity of *Spirulina* sp. is illustrated in Figure 2, while the rate of biomass and the specific growth rate are presented in Table 2.

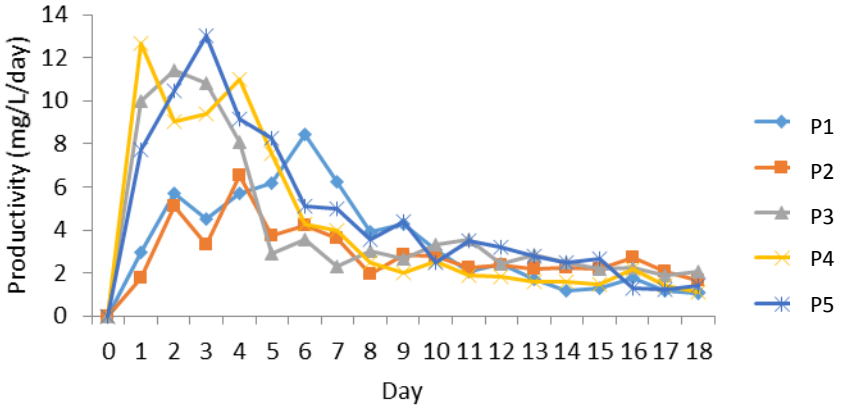

Figure 2. Productivity of *Spirulina* sp. of all treatments.

Table 2. Rate of biomass and specific growth rate of *Spirulina* sp. of all treatments.

Treatment	*Rate of biomass (mg/L)*	*Specific growth rate (%/day)*
P1^{c}	262.968	*27.3*
P2^{d}	218.765	*22.4*
P3^{b}	265.846	*34.2*
P4^{bc}	264.413	*31.2*
P5^{a}	315.449	*30.6*

a, b, c, d: significantly difference in Duncan test; same characters means no significantly difference

The productivity of *Spirulina* sp. was relatively high at D-1 to D-6, then decline to the end of observation. Treatment P3 showed the highest and P2 showed the lowest value. The result of ANOVA showed that the growth of the species was significantly different among treatments. The further analysis showed that all of treatments were responded significantly different from P5 as control, while between P1 and P3, then P3 and P4 showed no significantly different.

The nutrients condition was suitable for the algal growth. There were clear relationships between biomass increasing and nutrients reducing ($P<0.05$). It showed that *Spirulina* sp. was able to utilize nutrients resource of each treatment, as its requirement.

The final value of efficient determination matrix showed that P1 is the most efficient, and P2 is the least (Table 3). Although the highest rate of biomass was showed by P5 as control, the other parameters gave less weight. Therefore, the final value of P5 was lower than P1. P3 and P4 had similar condition, additionally.

Table 3. Final value of efficient determination matrix

Perlakuan	Value
P1	72
P2	50
P3	64
P4	54
P5	62

DISCUSSION

Ford and William (1989) defined karst as an area with specific condition as consequence of rocks solubility. Indonesian karst area is about 15.4 million hectare, and some of them are laid in West Java, especially Bogor District. One of karst area that has unique spring water is Ciseeng (Yoga, 2011).

Ciseeng karst water is a unique freshwater that has a high mineral content, both composition and concentration with relatively high temperature for about 40°C. The rich mineral condition comes from calcite rock and dolomite leaching karst sediment (Gushilman, 2010; Nurokhmah, 2015). Even in Ciseeng karst water, the leached mineral could raise the water salinity up to 30-35‰.

Karst water characteristic, especially The Ciseeng karst water, has some similarity to sea water that presumable suitable for the growth of marine microbiota, such as microalgae. Microalga is one responsive community to the change of water quality (Thakur *et al*., 2013).

Spirulina sp. is a widespread microalga that could be found in freshwater, brackish, or marine waters. It also commonly grows in warmwater lakes or ponds of tropical area (Tietze, 2004 in Wulandari, 2013). *Spirulina* sp. oftently shows dominant in bloom occurence (Ciferri, 1983 in Hu, 2004b). The inoculum of *Spirulina* sp. in this experiment was characterized as brackish to marine group that can live in 15–20‰ salinity of medium. The *Spirulina Medium* (SM) as manufactured artificial culture medium give specific condition of salinity (20‰) and pH (10) (Aiba and Ogawa, 1977).

The difference of *Spirulina*'s growth rate of each treatment was influenced by different medium condition. The lesser of SM in modified karst water, the lower pH of the medium. Therefore, the lowest pH was found in treatment P1 or medium with purely karst water.

The result showed that *Spirulina* sp. could live in all treatments medium, although several of the medium did not provide optimum condition for the microalga. Santosa (2010) and Rafiqul *et al*. (2005) explained that *Spirulina* sp. will grow well in medium with optimum condition of temperature (37°C), light intensity (2500 Lux), and pH (10). The temperature and light intensity of all treatments were controllable. Meanwhile, the pH was depended on the addition of SM. On the other hand, *Spirulina* sp. showed ability in utilizing nutrients in all treatments. Ammonium, nitrate, and nitrite, and also orthophosphate are required major nutrients for microalgae. Besides, it was shown that the declination phase was not arise yet at all treatments.

The growth pattern of *Spirulina* sp. of all medium treatments showed inclination to the end of observation. Theoretically, the growth pattern of microalgae in batch culture consists of lag, exponential, declination, stationer, and death phases (Fogg, 1975 in Santosa, 2010). All the way through the growth phases depends on environment or culture medium condition (Chainapong *et al.* 2012). *Spirulina* sp. started the exponential phases in one day culture period, and ended at different time. This was equal with the experiment result of Suantika and Hendrawandi (2009). The exponential stage is characterized by high rate of biomass increasing (Fogg and Thake, 1987 in Setyaningsih *et al.,* 2011). In this experiment, the microalga was not enter the stationer and death phases. The rate of biomass and growth rate was insignificantly different to nutrient modified karst water medium ($P>0.05$). It was allegedly related to the adequate nutrient of all treatments. It means that even pure karst medium, *Spirulina* sp. could utilize the available nutrients in the medium.

The determination matrix that was used as approach to choose the efficient culture medium resulted the rank of final values. The determination was based on the combination of weight and score of used parameters. The highest final value was determined for purely karst medium (treatment P1). This decision becomes an answer that karst water is potential to be an alternative medium to grow saline water microalgae in fresh water condition.

CONCLUSION

The productivity of *Spirulina* sp. that grown in purely karst water medium was as high as in nutrient modified karst water medium.

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Biobriquettes Potential from Cassava Leather, Plastic Low Density Polyethylene and Sludge from Industrial Waste Water Treatment Plant

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ABSTRACT

This research aimed to determine the best composition based on briquette compressive strength test where the test results determine suitability with SNI 01-6235-2000 about the quality requirements of wood charcoal briquettes. Best compositions were tested the quality of calorific value, moisture, volatile matter and ash content. The method in searching for the best composition by compressive strength test. Briquettes based on the best compressive strength was the composition of the carbonation (K3) was 52.88 kg/cm^2 and the composition of non carbonation (NK2) was 49,23 kg/cm^2. Test for briquettes selected for parameter carbonation briquette moisture content (K3) was 0.24% and non-carbonated briquettes (NK2) was 2.97%. Volatile Matter carbonation briquette (K3) was 42.74% and non carbonation briquettes (NK2) was 49.1%. Ash content carbonation briquette (K3) was 3.72% and non-carbonated briquettes (NK2) was 2.8%. Calorific value carbonation briquettes (K3) was 5061 calories / gram and non carbonation briquettes (NK2) was 4887.5 calories/gram. Total votes carbonation briquette (K3) was 93 and non carbonation briquettes (NK2) was 83. best briquette was K3 with fulfillment parameters that meet the highest quality requirements SNI 01-6235-2000 of charcoal briquettes.

Keywords: Biobriquette, cassava leather, plastics LDPE, sludge, total votes.

INTRODUCTION

The growth of energy consumption in Indonesia has reached about 7% per year dominated by fossil-based energy (95%). Fossil-based energy dependence condition will endangered national energy security. Therefore, renewable energy began to be developed in line with the limited reserves of fossil energy and the impact caused as air pollution, water pollution and other negative impacts on the environment (ESDM, 2011). Various solutions have been offered include utilizing renewable energy such as biomass which is converted into bio-coal briquettes (Hambali et al., 2007). The cassava leather can be used for briquettes because it has a fairly high calorific value, ie 4631 cal/g nearing SNI 01-6235-2000 of Quality Wood Charcoal Briquette, with a standard calorific value of briquettes is 5000 cal/g (Hayati, 2008).

Organic materials derived from residual waste of industrial treatment, such as sludge from WWTP PT Petrowidada, can be applied as a mixture of the raw material for making briquettes. This sludge derived from sludge dryer tub, which is largely a biomass containing organic material by up to 52.80% (Pinatih, 2015). In addition to the reduce of plastic waste generation, its additions as compotition of briquettes is also used to increase the calorific value. LDPE plastic has a high calorific value, ie 11095.24 cal/g (Warmadewanthi et al., 2010).

The purpose of this study is to get the appropriate composition and qualified characteristics of the briquette from a mixture of LDPE plastic waste, wastewater sludge and cassava peel which compare with

parameters of compressive strength, calorific value, moisture, volatile matter and ash content refers to SNI 01-6235 -2000 of Quality of Wood Charcoal Briquette.

MATERIAL AND METHODS

The method in this research is the briquettes that have been produced will be tested, first is to test the compressive strength based ASTM D-2166-66. Secondly, from the results of these tests, best briquette will be tested for calorific value (ASTM D 2015-96), the water content (ASTM D 3173-03), volatile matter (ASTM D 3174-02), and the ash content (ASTM D 3175-02). Then, scoring method is used to determine best qualified of briquettes. Assessment is done by entering a value for each parameter in the assessment table. Variations in the composition of the briquettes can be seen in Table-1.

Table 1. Various composition of briquettes.

No.	Briquettes Label	Composition of Briquettes
1.	NK1	32% LDPE, 48% Sludge, 20% Cassava peel
2.	NK2	24% LDPE, 36% Sludge, 40% Cassava peel
3.	NK3	16% LDPE, 24% Sludge, 60% Cassava peel
4.	K1	32% LDPE, 48% Sludge, 20% Cassava peel
5.	K2	24% LDPE, 36% Sludge, 40% Cassava peel
6.	K3	16% LDPE, 24% Sludge, 60% Cassava peel
7.	CONTROL (K & NK)	100% Cassava peel
		100% Sludge
		50% Cassava peel : 50% Sludge

Note: NK: Non Carbonation, K: Carbonation

RESULT AND DISCUSSION

Compressive Strength Test

The value of compressive strength test of the entire composition on briquette controls the main ingredient that has the best compressive strength of the cassava peel, the briquettes Non Carbonation (NK) 100% cassava peel has a strong value amounted to 49.22 kg/cm² while the briquettes Carbonation (K) 100% of Cassava peel 49.375 kg/cm². Strength of briquettes is important to be known as briquettes that will give will benefits during transport and storage (Triono, 2006).

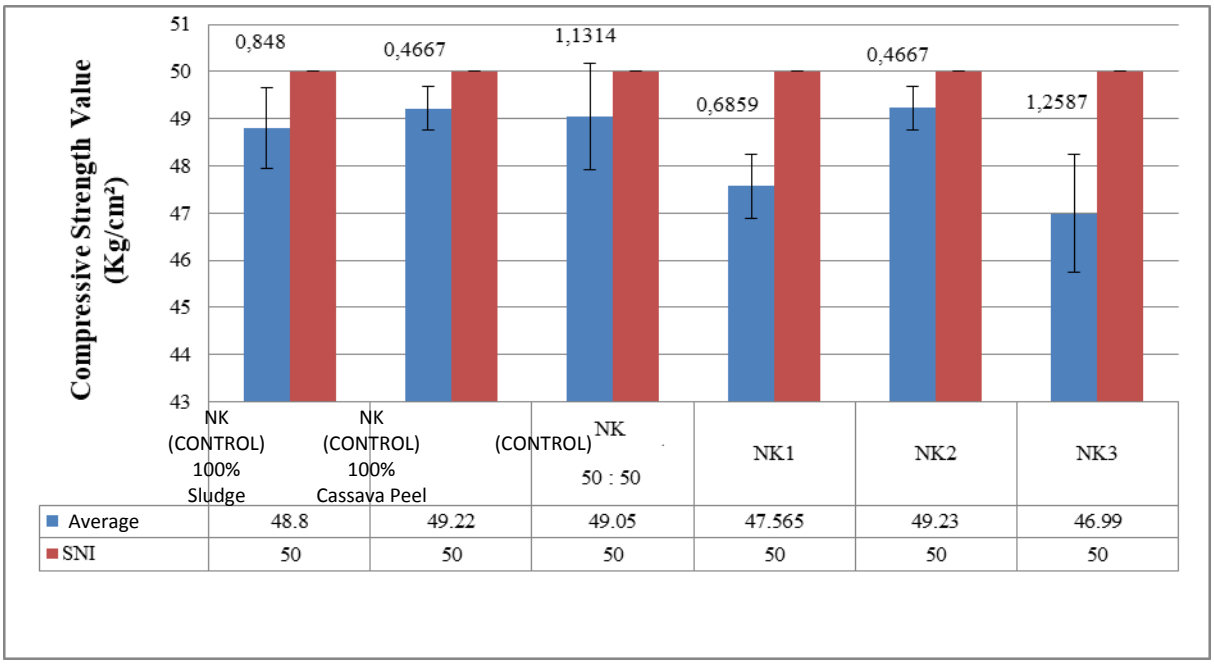

Figure 1. value of non carbonation compressive strength briquettes.

Figure 2. Biobriquettes.

Fig. 1 shows a non carbonation briquette best compressive strength 49.23 kg/cm² which NK2 composition with a mixture of 24% LDPE plastic, 36% sludge, and 40% cassava peel, cassava peel based on compositions number more than the amount of plastic and mud, so that the compressive strength to be good enough though not meet the quality requirements SNI 01-6235-2000 about briquettes at 50 kg/cm².

Figure 3 shows the best carbonation briquette compressive strength compressive strength of 52.88 kg/cm², which is the composition of the K3 with a mixture of 16% LDPE plastic, 24% sludge, and 60% cassava peel. More cassava peel will increase the compressive strength rises, cassava peel contains cellulose which is quite high by 5% (Nurlaili et al., 2013). The higher the content of cellulose in biomass, the biomass briquette compressive strength will be higher because the cellulose has elastic properties and is not easily broken (Riyanto, 2009). The compressive strength of briquettes K3 meet quality requirements SNI 01-6235-2000 about briquettes, which amounted to 50 kg/cm².

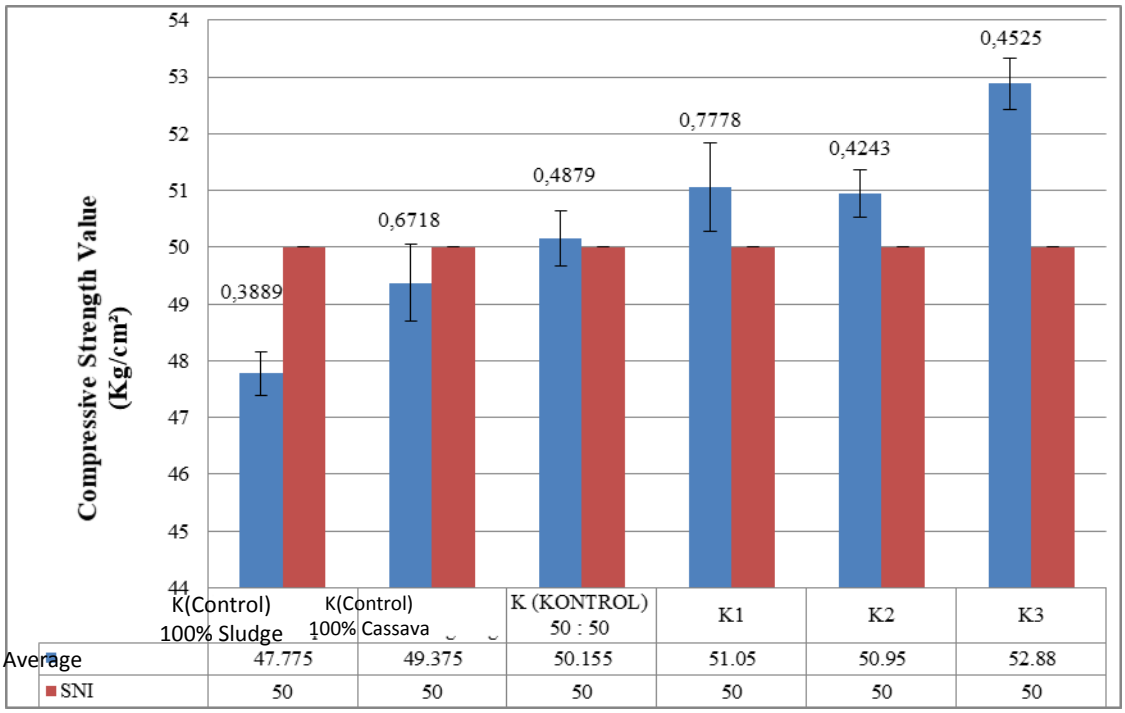

Figure 3. Value of carbonation compressive strength briquettes.

Calorific Value

Elected biobriquettes by best compressive strength tvalue is tested again with a calorific value parameter, briquettes are NK2 and K3. Figure 4 shows the K3 briquettes have a high calorific value, amounting to 5061 cal/gram, while NK2 briquettes have a lower calorific value of 4887.5 cal/gram. K3 briquettes have a calorific value that meet qualification of ISO standards 01-6235-2000, which stated that the minimum calorific value is 5000 cal/gram. The calorific value of the briquettes carbonation higher when compared with briquettes of non carbonation due to the carbonation process, materials, briquetting can easily release the volatile matter resulting calorific value higher and produced a clean burning with little smoke (Tobias et al., 2007).

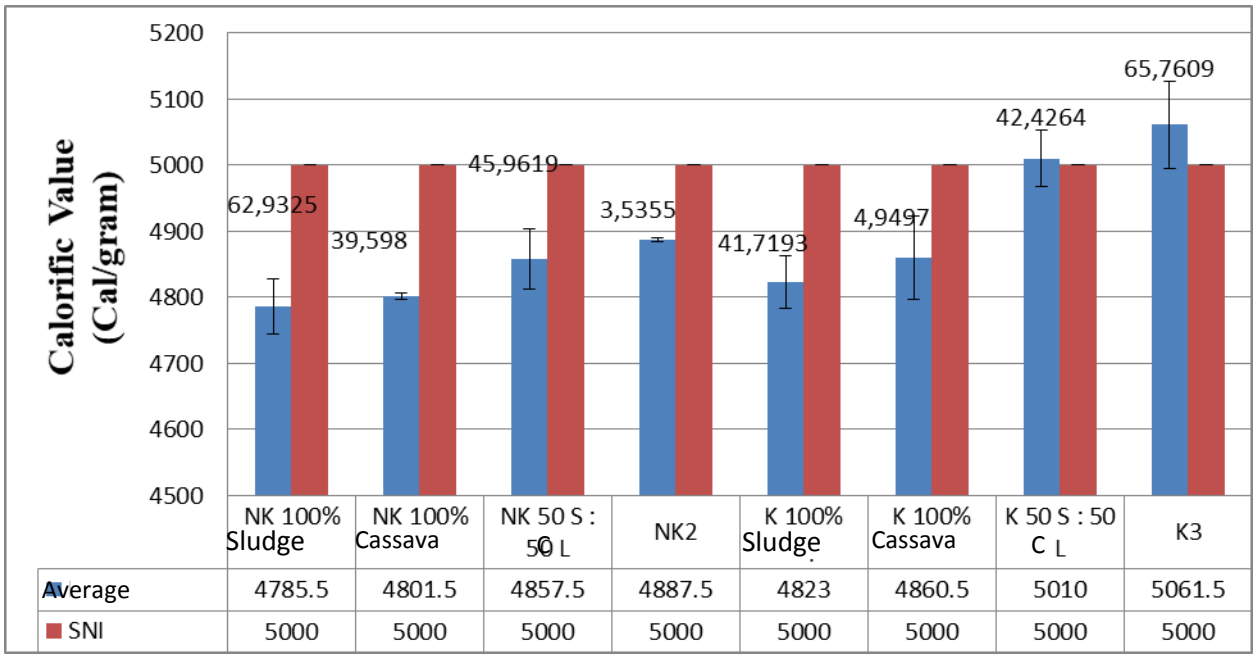

Figure 4. Calorific value of briquettes non carbonization and carbonation.

The heating value is an important parameter after the compressive strength as we can understand and measure the energy content of each fuel mass consumption has to generate a certain energy can be calculated precisely (Patabang, 2011). The calorific value of which has been obtained subsequently incorporated into the ratings or scoring column in Table 2. The calorific value of non carbonation briquette compositions (NK2) amounted to 4887.5 cal/gram, these values into the interval value of 4000-4499 cal/gram to obtain a category value / weight value of 54. the same principle is used for the assessment of carbonation briquette (K3) K3 briquettes have a calorific value of 5061 cal/gram that go into the interval > 5000 calories / gram to obtain a weight value of 60.

Table 2. Calorific value scoring.

No.	Range of value	Range of Calorific Value (cal/g)	Explanation
1.	60	>5000 kal/g	Value will be rounded up if the obtained value ≥ 0.5 for each treatment, and vice-versa
2.	54	5000-4500	
3.	48	4000-4499	
4.	42	3500-3999	
5.	36	3000-3499	
6.	30	2500-2999	
7.	24	2000-2499	
8.	18	1500-1999	
9.	12	1000-1499	
10.	6	<1000	

Water Content

Figure 5 shows the variation of the composition of briquettes value NK2 and K3, can be seen the value of the test states that the briquettes NK2 and K3 has a low water content. K3 has a composition of 16% LDPE plastic, 24% sludge, and 60% cassava peel has a water content of 0.2413% and briquettes NK2 having a composition of

24% LDPE plastic, 36% sludge, and 40% cassava peel with water levels 2, 9747%. Briquettes with carbonation process has a water content lower than the non-carbonated briquettes. Low levels of water due to the authoring process plays an important role in reducing the moisture content of briquettes (Sudarsono, 2010).

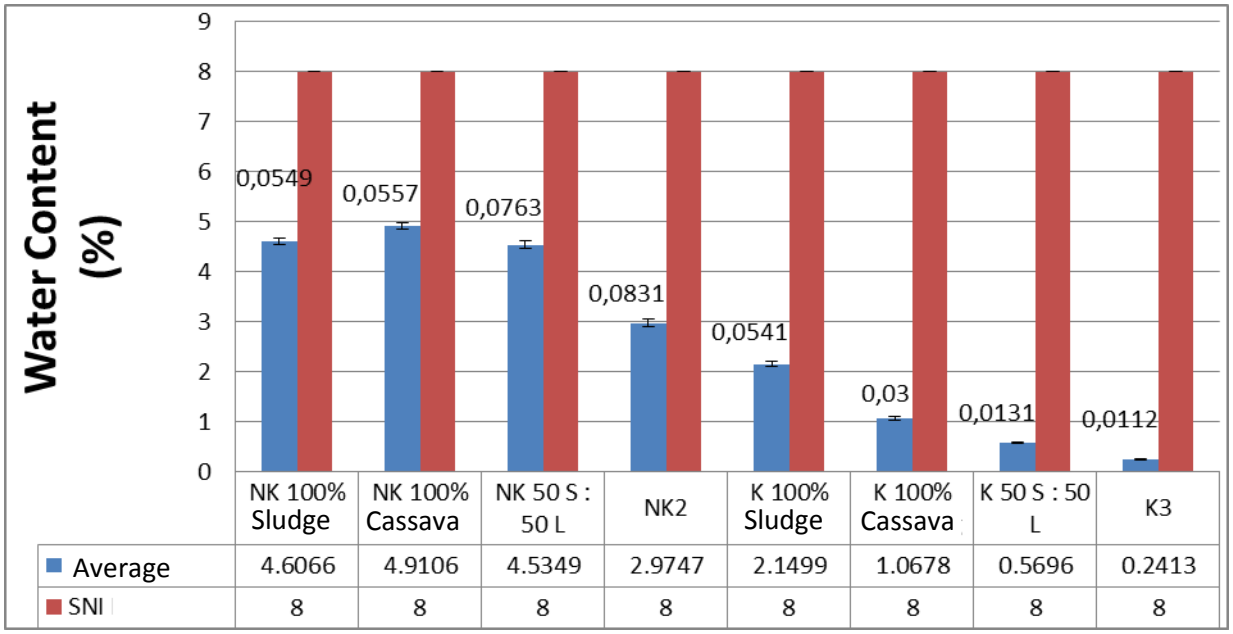

Figure 5. Moisture of non carbonization and carbonation briquettes.

The water content test in the briquettes is critical because water content affect the quality of the charcoal, the high water content will cause low heating value so that the quality of charcoal briquettes decline. A high water content and low calorific value cause the briquettes difficult to be lit or burned (Triono, 2006).Water content which has been obtained subsequently incorporated into the ratings or scoring column in Table 3, the water content value of non carbonation briquette composition (NK2) of 2.9747%, these values into the interval values of 3 to obtain the category of value / weight value by 14 . the same principle is used for the assessment of carbonation briquette (K3), K3 has a value of 0.2413% moisture content that goes into the interval> 1% thus obtained weight value by 20.

Table 3. Water content scoring.

No.	Range of Value	Water Content (%)	Explanation
1.	20	<1	Value will be rounded up if the obtained value ≥ 0.5 for each treatment, and vice-versa
2.	18	1	
3.	16	2	
4.	14	3	
5.	12	4	
6.	10	5	
7.	8	6	
8.	6	7	
9.	4	8	
10.	2	>8	

Volatile Matter

Figure 6 shows the value of briquettes K3 which has a composition of 16% LDPE plastic, 24% sludge, and 60% cassava peel has a volatile matter content value of 42.5031% and briquettes NK2 having a composition of

24% LDPE plastic, 36% sludge, and 40 % cassava peel with volatile matter content of 46.1281%. Volatile matter of NK2 and K3 in accordance with the control of volatile matter content test that shows the basic ingredients are carbonated briquettes have a volatile matter content is lower than non carbonation. High volatile matter is influenced by the volatile substances contained from these materials. The higher the volatile matter, then the ignition timing will be longer and faster burning time (Sulistyanto, 2000). Another factor influencing the plastic is the addition of LDPE, LDPE plastic accounted volatile matter content of the total of 99.73% (Warmadewanti et al., 2010). Volatile matter content value briquettes NK2 and K3 does not meet the quality requirements SNI 01-6235-2000 of briquettes by 15%.

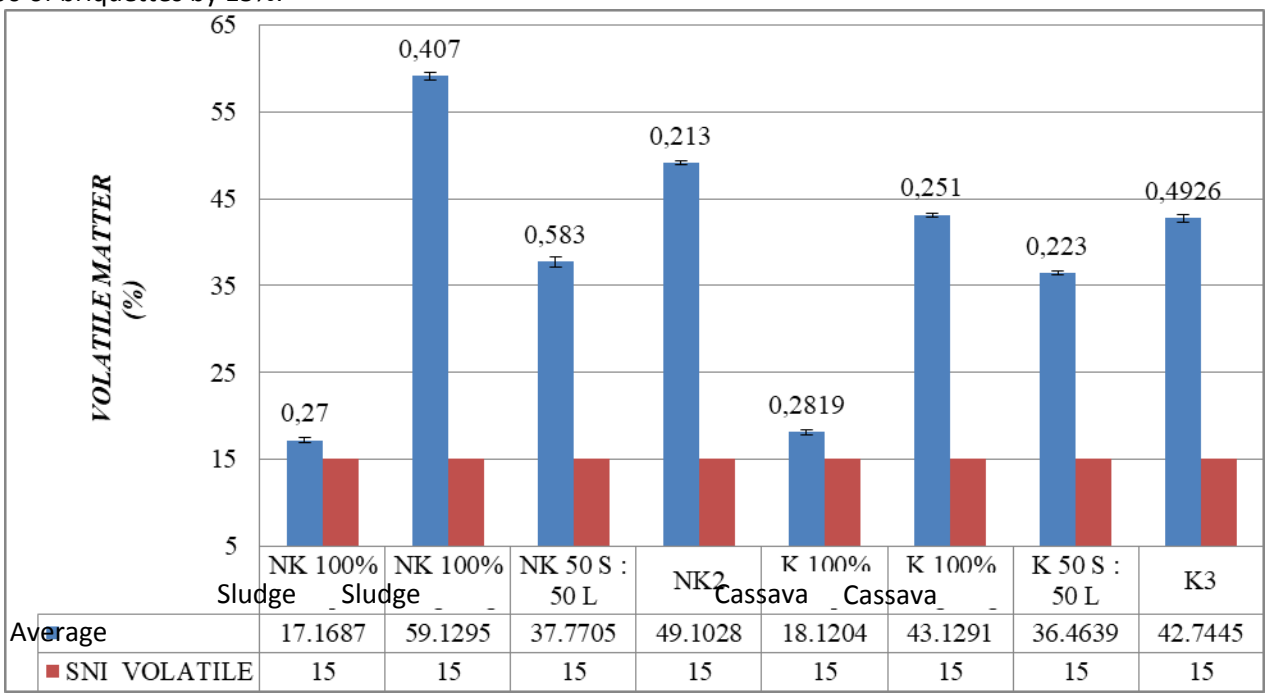

Figure 6. Non-volatile matter briquettes carbonization and carbonation.

Briquettes which have been analyzed volatile matter content is then performed scoring process parameters in accordance with Table 3, for briquette K3 with a value of 42.7445% volatile matter content gets a score of 1, while for briquette NK2 with volatile matter content of 49.1028% earn score 1 vote or scoring aims to determine the best value between briquettes accumulation of carbonated and non carbonated.

Table 3. Volatile matter scoring.

No.	Value of Category	*Volatile Matter*(%)	Explanation
1.	10	<7	Value will be rounded up if the obtained value ≥ 0.5 for each treatment, and vice-versa
2.	9	7,5-8	
3.	8	8,5-9	
4.	7	9,5-10	
5.	6	10,5-11	
6.	5	11,5-12	
7.	4	12,5-13	
8.	3	13,5-14	

Ash Content

Ash content briquettes NK2 and K3 showed both the briquettes have a content value is quite low ash content. Briquettes carbonation (K3) which has a composition of 16% LDPE plastic, 24% sludge, and 60% cassava

peel has a value of 3.7229% ash content and briquettes of non carbonation (NK2) which has a composition of 24% LDPE plastic, 36% sludge, and 40% cassava peel with ash content of 2.8034%. Briquettes with non carbonation process has a value lower ash content than the briquettes carbonation, these values correspond to control ash content test that shows the basic ingredients of non carbonation briquette which has a lower ash content of the karbonation.

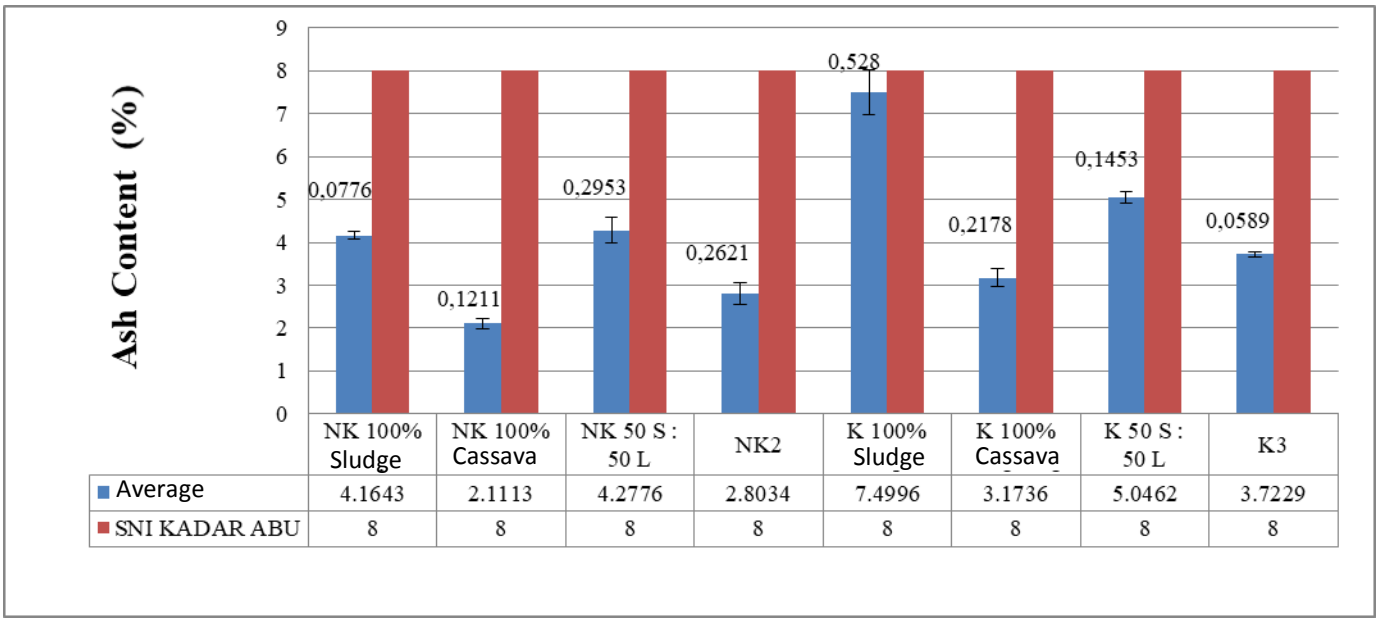

Figure 7. Ash content of non carbonization and carbonation briquettes.

Carbonization treatment in a sample can eliminate volatile matter content and increase the levels of carbon bound in the sample, carbonization can also increase the levels of ash in the sample (Warmadewanti et al., 2010). The tendency for the ash content briquettes decreases were also seen with increasing mass percentage of cassava peel in briquettes. This is due to the ash content of cassava small skin, which is 3.1736% of the sample carbonation and 2.1113% in a sample of non carbonation. The addition of cassava skin is what causes the ash content is low. Briquettes that have analyzed the value of the ash content is then performed scoring process parameters in accordance with Table 4, for briquette Carbonation (K3) with ash content of 3.7229% scored 6, while for briquette Non Carbonation (NK2) with ash content of 2.8034% get a score of 7, or scoring assessment aims to determine the best value between briquettes accumulation of carbonated and non carbonated.

Table 4. Ash content scoring.

No.	Range of Value	Ash Content (%)	Explanation
1.	10	<1	Value will be rounded up if the obtained value ≥ 0.5 for each treatment, and vice-versa
2.	9	1	
3.	8	2	
4.	7	3	
5.	6	4	
6.	5	5	
7.	4	6	
8.	3	7	
9.	2	8	
10.	1	>8	

CONCLUSION

The conclusion of this research are:

1. The value of non carbonation briquette compressive strength (NK) 100% sludge, 100% leather cassava, 50:50, NK1, NK2, and NK3 sequentially 48.8 kg / cm², 49.22 kg / cm², 49.05 kg / cm², 47.565 kg / cm², 49.23 kg / cm², and 46.99 kg / cm². Carbonation briquette compressive strength value (K) 100% sludge, 100% leather cassava, 50:50 mud, K1, K2, and K3 sequence of 47.775 kg / cm², 49.375 kg / cm², 50.155 kg / cm², 51.05 kg / cm², 50.95 kg / cm², and 52.88 kg / cm².
2. Briquette NK2 and K3 is a composition with the compressive strength selected. NK2 has a calorific value of 4887.5 cal / g, the water content of 2.97%, 49.1% volatile matter and ash content of 2.8%. K3 briquettes have a calorific value of 5061 cal / g, the water content of 0.24%, volatile matter 42.74%, and the ash content of 3.72%.
3. Briquette briquette K3 is the best because it has a total value of 87 and have parameters that meet the highest quality requirements SNI 01-6235-2000 about briquettes.

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Pattern of Nucleotides Subtitution of *CpTI* Gen in Some Plantsas Teaching Material of Molecular Genetics for Biology Education Students

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ABSTRACT

CpTI gene is one of plant gene that plays an important role in synthesis trypsin inhibitor protein from biotic and abiotic stress. The exploration of CpTI gene sequences have been reported by some previous studies, especially in Fabaceae. At NCBI, data base TI genesencode proteins of genus Vigna and Phaseolus. However, the learning process in Biology Education of UNPGRI Kediri did not use genomic database at NCBI yet. Gene sequencesused tend to be without basic prediction of protein structures in which it will be formed from amino acid sequences produced from the gene sequence. These conditions led to misunderstanding of the concept of a functional gene sequences to the students. Moreover, driven by this condition, the current aim is to describe the use of pattern of nucleotides substitution of *CpTI* genes in some plantsas teaching material of molecular genetics for biology education students. Furthermore, the sequences database exploration of nucleotide CpTI gen were done based Fatchiyah and BioEdit Sequence Aligment Editor program was used to analyze nucleotide substitution pattern of CpTI gene (transversion or transition). Feasibility of the study was done by some Biology Education practitioners to determine whether the product can be used to improve molecular genetic lecturing or not. The results of database exploration obtained main information that there were 9 CpTI gene in Vigna unguiculata (ID gene: 50659116, 90101503, 45934292, 28569583, 28569585, 16555416, 28569591, 28569589, 28569587) and there were 15 sites of nucleotide substitution i.e. 12 sites transition substitution and 3 sites transversion substitution. In addition, CpTI gene sequences used to explain the concept of nucleotide changes, obtained feasibility up to 89. It means that database exploration can be used to improve molecular genetic lecturing to describe the pattern of nucleotide sequence.

INTRODUCTION

One of the topics that are taught in genetic is a mutation. Mutation is the process that produces the changes in the structure of DNA or chromosomes. Mutations in the DNA involve an addition, deletion and substitution of DNA structure or chromosomes. The main case of mutation is the substitution at the third codon position. The percentage of base substitution in this positionis more than 50% of all mutations (Amin, 2003). Hence, knowlegde of the pattern of nucleotide substitution is important both to our understanding of molecular sequence evolution and to reliable estimation of phylogenetic relationships (Yang, 1994). There are two kinds of nucleotide changes in the gene encoding a protein that is a change that will affect the primary structure of the protein encoded (nonsynonymous substitutions) and changes that do not affect the protein encoded (Synonymous substitutions). Mutations that produce amino acid replacement has a high possibility to eliminate the function of a protein if the mutation occurs in sitenonsynonymous. Consequently, the majority of nonsynonymous mutations will be eliminated from the population through selection of purification. The result

will be a decrease in the rate of nonsynonymous site. Conversely, changes to the site Synonymous also more likely to be neutral and numbers will continue to grow in population (Graur and Wen-Hsiung, 2000).

However, the learning process of genetic in UN PGRI Kediri did not run successfully yet. The students had misconception dealing with genetic. This condition was caused by some learning obstacles.The first is unavailability of good and relevant teaching material. Most of handout content still describes about Mendel genetics and molecular genetics. The second, learning materials for practicum do not support the concept yet. Students were provided with learning materials about simple genome isolation, Mendel'scrossobservation of fruit fly's chromosome. During conducting simple genome isolation, lecturer or instructor did not give enough explanation about DNA isolation yet. The practicum of Mendel's cross used button whereby it unable to show the characteristics of dominant or recessive genetic.Meanwhile, the procedure of the observation of chromosome of fruit fly conducted inappropriately yet. The third is irrelevant media for analyzing genomic. The last is learning process do not use the development of genomic database (Primandiri and Santoso, 2015).

In addition, the evaluation of Genetic instructional materials used in Biology education in UN PGRI revealed that the handout needs to be revised (mayor revision). It also needs to be discussed about molecular genetics. The most important finding that relevant with the study on learning process of genetics is the handout needs to be provided with various genetics examples that areavailable in a gen bank database in order to give contextual examples and to provide a relevant concept (Primandiri *et al*., 2016).

Driven by the findings of previous researches, the current research investigates the use of examples of gen that are available in a gen bank database.Furthermore, gen that is selected is *CpTI*. It is selected due to some reasons. The first, to learn more about the nucleotide substitution pattern, can be done with *CpTI* gene sequences.The second, *CpTI* gene is one of plant gene that play important role in synthesis trypsin inhibitor protein from biotic and abiotic stress (Ghosal *et al*., 2001; Xu *et al*., 1996; Ismail *et al*., 2010). Trypsin inhibitor proteins are known to have an important role in plant defense system against predators and pathogens (Ismail *et al*., 2010). This protein is involved in the digestive process of insects that cause a reduction in amino acids in their growth and development (Fan and Wu, 2005), so as to nutritional deficiencies that become stunted growth and development. The last, the exploration of CpTI gene sequences have been reported by some previous study especially in Fabaceae. At NCBI, data base TI genes that encode proteins of Vigna and Phaseolus. In general genomic data available from the isolated DNA sequences of varying lengths each genus include 572 bp, 663 bp, 340 bp and 326 bp that CDS sequences also vary in each genus include 302 ...> 572, 242 ... 565, 44 ... 340, 2 ... 325 and 355 ... 838 (Primandiri *et al*., 2016). Finally, this research is intended to describe the use of pattern of nucleotides substitution of CpTI genes in some plants as teaching material of molecular genetics for biology education students.

METHOD

In line with the aim of the research, descriptive qualitative was selected as research design. Sequences database exploration of nucleotide *CpTI* gene were done based Fatchiyah (2014) and *BioEdit Sequence Aligment Editor* program was used to analyze of nucleotide substitution pattern of *CpTI* gene (transversion or transition). Finally, feasibility study was done by some Biology Education practitioners to determine whether the product can be used to improve molecular genetic lecturing or not.

RESULTS AND DISCUSSION

The result of gen exploration inNCBIobtained main information that there were 9 CpTI genes in *Vigna unguiculata* (ID gene: 50659116, 90101503, 45934292, 28569583, 28569585, 16555416, 28569591, 28569589, 28569587). Moreover, the result of alignment found that there were 15 sites of nucleotide substitution i.e. 12 sites transition substitution and 3 sites transversion substitution. The example of transition substitution pattern

was found on number sites 204 in ID gen 28569585 (G to A) and transversion substitution was found in number sites 149 (T to A) can be seen in the figure 1.

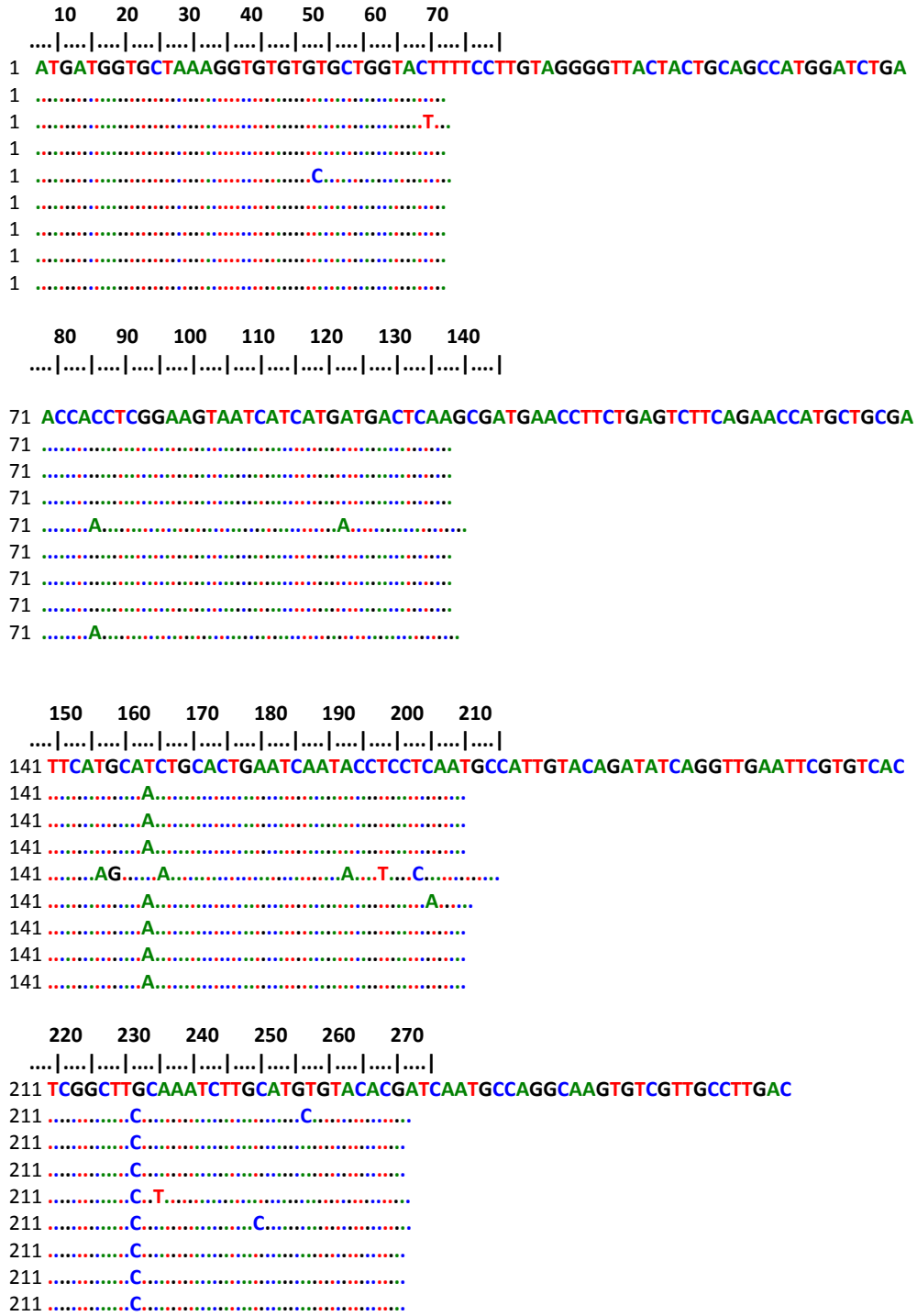

Figure 1. Figure alignment CpTI gene.

Furthermore, the result of this alignment was used as learning materials in mutation. However, before this material is used to teach mutation, the assessment was done by three practitioners to evaluate whether the

alignment is relevant to be used as the real example to explain the materials of mutation. The following table shows the result of alignment evaluation conducted by practitioners.

CpTI gene sequences used to explain the concept of nucleotide changes, has been obtained feasibility up to 89. It means that database exploration can be used to improve molecular genetic lecturing to describe the pattern of nucleotide sequence. Sequences used enable to explain nucleotide changes appropriately and efficiently to improve the understanding of learners. In addition, it does not induce misconceptions and correspond with the latest scientific developments.

Table 1. Assessment by the practitioner to instructional media.

No.	Aspects of Assessment	score practitioners 1	score practitioners 2	score practitioners 3
1	Provide basic information of a material	4	4	4
2	Aroused the interest of learners to learn	4	5	5
3	Presents a problem to be discussed in groups	4	4	4
4	Providing sequences derived from the gene bank to clarify the matter	5	5	4
5	Mutations can be determined clearly	5	5	5
	Acquisition score	22	23	22
	Final score	88	92	88
	Mean		89	

When genomic database at NCBIwas not used in the learning processyet, gene sequences were made examples in learning process tend to be without basic prediction of protein structures whereby it will be formed from amino acid sequences produced from the gene sequence. These conditions led to students' misunderstanding about the concept of a functional gene sequences. The development of a genetic data base can be used by lecturers as an example of real (based on research) to give the correct concept. Lecturers can download a functional gene sequence (without introns) at NCBI. Lecturers can give examples of successful sequences studied previously. Through the example of the sequence, can be obtained predicted amino acid sequence is correct so that construction modeling protein structures in three dementionalnya definitely known correctly (Primandiri *et al*., 2016).

CONCLUSION

There are many gene sequences are available in gen bank database whereby these can be used to teach mutation. Moreover, the result of this research shows that the use CpTI gene sequences can be use to explain the concept of nucleotide changes. In addition, the database exploration can be used to improve molecular genetic lecturing to describe the pattern of nucleotide sequence. In sum, the use of pattern of nucleotides substitution of CpTI genes in some plants genetics is relevant to be usedas teaching material of molecular for biology education students.

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Genetic Variation Analysis of Local Durian (*Durio Spp*.) of *Ex Situ* Hortimart Collection Bawen Based on Microsatellite Dna Marker

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ABSTRACT

Indonesia has the biggest center of durio cultivar diversity in the world and high species diversity. This research is to identify the allelic diversity of 41 accessions of the local durians in Hortimart Central Java based on microsatellite markers. The methods used in this research were: DNA Isolation (modified CTAB method), DNA amplification on three microsatellite locus, Amplification product will be separated using PAGE and DNA visualization using silver nitrate staining. The result showed that three microsatellite locus has 92 alleles with an average of alleles per locus was 30.6. One locus showed more than two alleles indicates that the durian is polyploid. The highest number of alleles found in mDz3D11 locus was 41 alleles. The frequency of allele were mDz3D11 (1,2-12,2%), mDz03H9 (1,2-13,4%) and mDz78B2 (1,2-12,2%). The parameter of *D. zibethinus* diversity using the microsatellite markers demonstrated high results and was able to detect specific alleles. The mDz78B2 locus detected 8 specific alleles, while the mDz3D11 locus detected 15 specific alleles. The relationship of allele diversities with cluster analysis by UPGMA method used NTSYS version 2.02. The kinship of the 41 local durian accessions was classified into 2 groups with the level of genetic similarity ranging from 0.72-0.97.

Keywords: *Durio, allele variation, microsatellite, ex situ collection.*

INTRODUCTION

Durian is a fruit commodity that has an importantly economic value in the world. Indonesia becomes the center of species diversity of durian, especially in Kalimantan (Uji, 2007). The diversity of durian classified into the genus *Durio* and the tribe *Bombacaceae* has approximately 27 species all over the world, 18 species in Borneo, 11 species in Malaysia, and 7 species in Sumatra. In other parts of Indonesia is only recognized one type of durian, namely *Durio zibethinus*. Most of these types of durian are endemic species to Borneo. The high number of *Durio* species growing in Kalimantan becomes evidence that the area is a central of the spread of durian family (Uji, 2005).

D. zibethinus is the most popular durian for consumption. The total of *D. zibethinus* varieties/cultivars in Indonesia (afterward they refer to local durians) are known to have ten or even hundred varieties of flavor, odor, texture, and flesh color (Uji, 2007). In general, those durian varieties have morphological features difficult to distinguish from each other. Their indistinct characteristics possibly result in mistakes in selecting local durians especially for commercial purposes. Morphological characteristics of durian are not always fixed because its phenotype is influenced by genetic factors and the interaction of genetic factors with the environment (Pandin, 2010).

The number of durian varieties in Indonesia is abundant, but their identity is not clear and there is no complete database about their morphological characteristics and other markers which are able to distinguish

exactly one variety to another. Information about superior local durians so far is just on their morphological characteristics so that the possibility of mistake in miscasting seed among durian varieties is quite strong. Information about their characteristics which promises precision and consistency can be obtained through DNA markers. Genetic diversity of local durian based on DNA markers in Indonesia is still very limited. Therefore, analysis of genetic diversity using DNA markers is important to uncover the richness of durian in Indonesia.

The diversity of variety with high degree of morphological likeness can be determined through DNA markers including microsatellite marker. Microsatellite is proved to be able to identify the diversity of some plants, including the germplasm diversity of mango (Zainudin *et al*., 2010; Pancoro, 2005), orange (Novelli *et al*., 2005) and apple (Patzak *et al*., 2012). On annual crops, microsatellites can also identify the varieties of wheat (Feng *et al*., 2006), rice (Rahman *et al*., 2009) and potatoes (Novakova *et al*., 2009). Microsatellites on plantation crops may also reveal the diversity of palm oil (Made & Sekar, 2013), teak (Imas, 2013; Nurtjahjaningsih & Rimbawanto, 2012).

Malaysia and Thailand have large-scale durian plantations with the best and superior cultivars in each of those countries. The cultivated durian varieties or the most superior result of plant breeding in both countries have been known to have definite and stable characteristics of superiority. Genotypes of superior durians in Thailand and Malaysia have been discovered and certainly can produce uniform pieces. In Indonesia, research on the genotypes of superior durian varieties has been recently done sporadically, but it has not covered wholly the richness of local durian in this country. Identification of alleles on specific microsatellite loci to ensure the genotypes of durian is one effective way to distinguish between one durian variety to another. Hortimart Agro Center in Central Java has a number of local durian collection some of which produce durian fruit which have superior characteristics. Analysis of local durian biodiversity using microsatellite markers need to be done to ensure the accession of local durian in Central Java especially in the Hortimart's collection to reveal molecular diversity as well as molecular characteristics which can be the marker of local durian.

MATERIAL AND METHOD

Plant Materials and Primers

A total of 41 durio accessions were a ex situ collection of the Hortimart Bawen in Central Java. Genetic variation among durio germplasm was investigated using three pairs of primarily microsatellites. The accessions were classified by UPGMA, to determine genetic relationship among the durio germpalsm.

DNA Extraction, Microsatellite Region Amplification and Silver Staining

Total DNA was extracted from fresh young leaves using a modified CTAB procedure (Vanijajiva, 2011). Annealing temperature of microsatellite regions was conducted by following Panca (2015) with modifications (Table 1), Amplification followed the procedure by Retnoningsih (2011) (Table 2) using thermal cycler (peqSTAR 2X) (Applied Biosystems®). PCR products were separated on 6 % denaturing polyacrylamide gels 6% containing 7 M of urea (Sigma-Aldrich Chemicals Germany®) at 45-60 watt for one hour. Alleles of each microsatellite were visualized by silver staining according to the procedure of Creste *et a*l. (2001) modified by Retnoningsih (2011). The size of each allele was estimated using 50 bp DNA ladder (Invitrogen®).

Table 1. Annealing temperature of Microsatellite

Locus	Product size	*Annealing* Temperature	Locus Sequence
mDz1G3	151	62,0^{0}C	F:5'-GTT AGT TCG TCG TTT CGG CT-3'
			R:5'-TAC GCG TGG ACT CAC AA-3'
mDz3D11	163	60,9^{0}C	F:5'- CAG CCC TGA CAT ATC CTG GT-3'
			R:5'- GCT TAC GCG TGG ACT AGA CT-3'
mDz03H9	171	60,9^{0}C	F:5'- AGC CTC CGT ATC TTT ACA TCG T-3'
			R:5'- CAT TCG ATGCTA CCA CAC CG-3'
mDz78B2	157	60,9^{0}C	F:5'- GCG TGG ACT AAC AAG TGG TA-3'
			R:5'- ATA TCA AGG GCA GTC TCG TG-3'
mDz1H32	266	57,4^{0}C	F:5'- AGC ACC ACT CAT ATG CCC AT-3'
			R:5'- TTG GCC GAT TCC TCT TGC TT-3'

*Based on personal communication by Panca (2015).

Table 2. Cycle of PCR microsatellite DNA

Procces	Temperature (^{0}C)	Time (menit)	Cycle
Pre-denaturation	94	40"	1
Denaturation	94	30'	
Anneling	60,9	45'	35
Extension	72	45'	
Final extension	72	5"	1

*Source: Retnoningsih *et al.* (2011)

Data Analysis

Genetic variation analysis of characterization was carried out based on the presence of microsatellite bands from each accession. Each band variant was treated as an allele. Different sizes of these alleles were estimated by a 50-bp ladder molecular size standard (Invitrogen life technologies Japan®). The alleles were scored for presence (1) or absence (0). Heterozygous genotype exhibited 2 alleles or more, while homozygous genotype exhibited only 1 allele. Matrixes of genetic likeness among those genotypes were calculated using the procedure of SIMQUAL (Similarity for Qualitative Data) Nei & Li (1979). The matrix of likeness was used to analyze agglomeration of Sequential Angglomerative Hierarcichal and Nested (SAHN), clustering by the method of Unweighted Pair-group Method with Arithmatic Averaging (UPGMA) and plotted in a dendrogram using the Tree Plot function of the NTSYSpc version 2.1 (Rohlf, 2000).

RESULT AND DISCUSSION

All microsatellite markers generated clearly distinguishable alleles and polymorphic patterns. A totall of 92 alleles were detected with the three markers. The number of alleles per locus varied from 13 to 41, with an average of 30.6 alleles per locus. The size of the alleles ranged from approximately 154 to 884. The highest degree of polymorphism was observed with mDz03H9 and the lowest was detected with mD78B2 (Table 3). The accessions were classified by UPGMA, presented in Figure 2.

Table 3. Alleles size, number of alleles, and frequency allele from analysis of 41 durio accessions using three microsatellite markers.

Locus	Allele size range (bp)	Number of allele	Frequency allele (%)
mDz78B2	166-882	38	1,2-12,2
mDz3D11	224-884	41	1,2-12,2
mDz03H9	154-410	13	1,2-13,4

Allele Variation of Local Durian Based on Microsatellite Marker

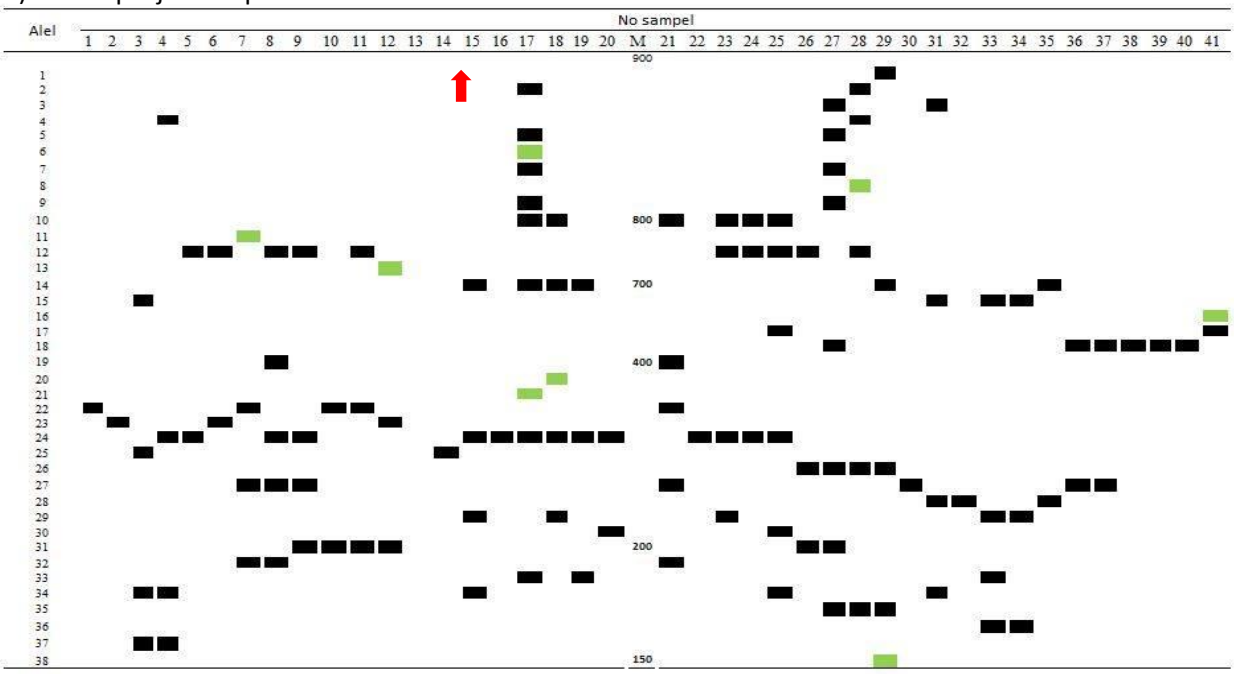
Each band of microsatellite DNA amplification products separated on polyacrylamide gel was a particular allele. Determination of appearing sizes was measured at the same rate and number of alleles calculated on homologous locus. Polymorphism of allele was shown through variations of allele at one specific locus. Three primers used to analyze the genetic diversity of local durian accession showed pattern of highly polymorphic band. Allele sizes were between 154-884 kb (Table 3). Microsatellite was an effective marker for detection of polyploidy or not (Bruvo *et al*., 2004). Loci of MDz03H9 showed one or two alleles at each accession. However locus mDz3D11 and mDz78B2 evenly showed 3-10 alleles for each accession so that it was included in a polyploid individual. According to Datta (1968) durian is said to be diploid, this may be just a karyotype study on certain cultivars which indeed showed 2n. Polyploidy was associated with morphological characteristics, geographical distribution and environmental conditions as a place of plants to grow. Polyploidy of plants can be determined by the acidity of soil for long time (Sengbuch, 2004). Diploid nature is caused by acidic growing area, while tetraploid nature is because of constantly alkaline environment (Sengbuch, 2004). Polyploidy durian in this study supposedly originated from the mother plant that came from the ground with alkaline pH. Naturally, the morphology of polyploidy plants are larger and robust than diploid plants, such as larger leaf surface, bigger flower organ, thicker stems and taller plants.

Polyploid cases can occur due to several factors among which are: an "*duplicated chromosomal regions*" that have occurred in the banana (Retnoningsih 2010; Creste *et al*. 2003, 2004; Kaemmer *et al*., 1997); Naturally occurring or artificial; Reproductive behavior (Stansfield, 1991); mutations (Oliveira *et al*., 2006); cross-pollination (Tasliah *et al*., 2013). Changes in environmental conditions that allow result in changes in ploidy also caused by the propagation of lineage and speciation events that may affect changes in the number of chromosomes (Sarah *et al*., 2000).

The evidence that occurs in nature, polyploid occur due to electric shock (lightning), extreme environmental conditions, or cross followed by disruption of cell division. Specific reproductive behavior conducive to a polyploid, eg vegetative propagation or parthenogenesis, and pervasive (Stansfield, 1991). Their incidence of mutations at microsatellite sequences allegedly caused the difference in length of microsatellite regions flanked by two primer forward (F) and reverse (R). The result of these mutations can be moved by the events of crossovers when the plants will reproduce (Oliveira *et al*., 2006). Cases have also occurred mango plant are diploid (Tasliah *et al*. 2013) as well as on plants self-pollinated, such as rice (Tasliah *et al*., 2011; Utami *et al*., 2011) and soybean (Chaerani *et al*., 2011) turns found polyploid alleles. In durian if done cross-pollination process allows the integration of several different areas microsatellite length in one individual. The events that have occurred tens or hundreds of years ago can be detected on molecular analysis.

Based on Figure 1 (a) locus allele mDz78B2 showed Null alleles. Null alleles at microsatellite loci can not be visualized because there is a mutation in the flanking sequences to complement with the primary, so this locus could not be amplified (Dakin & Avise 2004). Null allele in the sample "*Jagal bilowo*" likely caused by mutations in the primary site or part of the DNA template attachment of a primer. This is in accordance with the opinion of Callen *et al.* (1993) which states that: 1) null alleles can occur because of changes in the nucleotide sequence (substitution and mutation) that can prevent paired primer on the DNA template. 2) because the sequence of nucleotide bases that make up the primer is not complementary to the base pairs that make up DNA genome, so that DNA can not be amplified during PCR. 3) In addition, the cause is slippage during PCR (Shinde *et al.,* 2003). Certainty null allele is evidenced by the PCR and electrophoresis.

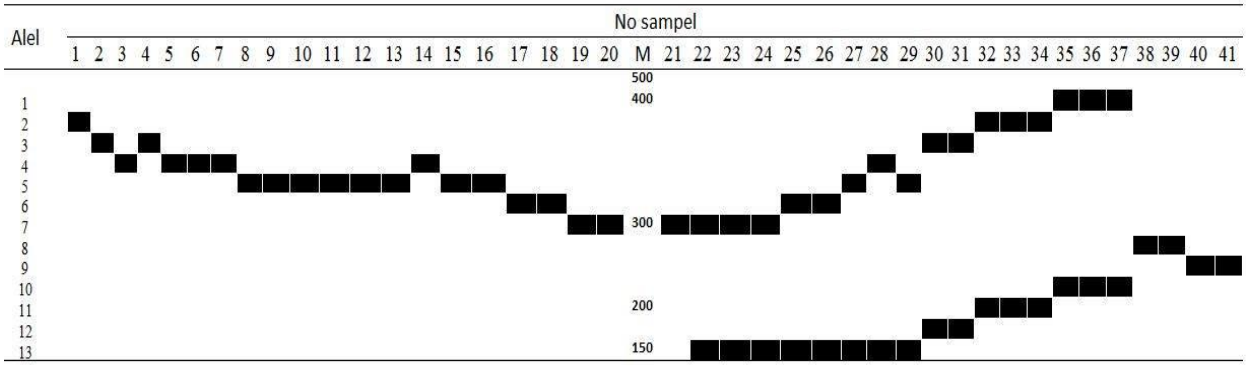
a) Allele projection profile of locus mDz78B2



Description: alleles (1-38) is a great diversity of alleles obtained by kb size difference.

= Alleles were detected; = Null allele ; = fic alleles

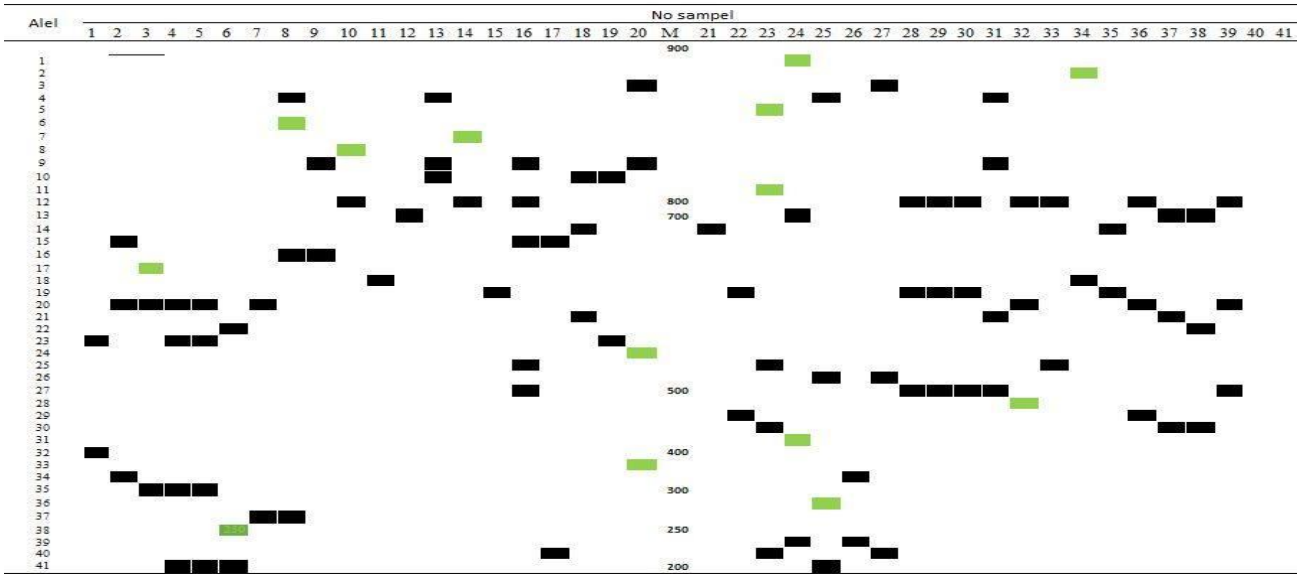
b) Allele projection profile of locus mDz03H9



Description: alleles (1-13) is a great diversity of alleles obtained by kb size difference.

= Alleles were detected

c) Allele projection profile of locus mDz3D11



Description: alleles (1-41) is a great diversity of alleles obtained by kb size difference.

= Alleles were detected; = Specific alleles

Figure 1. Projection DNA microsatellite after separated using 6% polyacrylamide gel.

Table 2 shows that the three microsatellite loci on the local durian in general, polymorphic, since it has an allele frequency less than or equal to 95%,. This is in accordance with Wandia *et al*. (2003) states that the polymorphic loci expressed as the number of alleles in the population at the locus of the more than one with the most common allele frequency less than or equal to 95%. However, more research needs to be done to ensure the genetic diversity of the population using microsatellite markers more. Takdir *et al*. (2009) suggest that molecular data depends on the number of primary election used.

Locus is the seat allele in chromosomes. Allele homozygous/heterozygous than one pair of genes occupying the same locus on the homologous chromosome pairs (identical). Therefore, the existence of specific alleles found at each locus is suspected of accession of the durian has a distinctive phenotypic properties because molecular markers often located near genes (Muladno, 2002). Specific alleles have been detected in the microsatellite loci used (Table 4). In mDz78B2 locus specific alleles known to many as eight and as many as 15 alleles mDz3D11. On accession have specific alleles, it is possible there are also specific morphological features. Locus mDz03H9 not detected any specific allele. Analysis using three microsatellite loci can detect durian 17 accessions of ex situ collections hortimart Bawen have specific alleles. Number of specific alleles would be more accurate with more loci analysis (Courtois, 2002). This is because the scattered microsatellite sequences in the genomes of plants (Solihin, 2000) and are often located within introns (Muladno, 2002), so that the specific alleles can be used as a marker character, especially at the local durian plant in Central Java. At locus mDz3D11 of 41 accessions durian analyzed 15 specific alleles detected. Therefore, it is expected both this locus may be a marker for the identification of accession durian widely.

Table 4. Allele-specific alleles were detected at 41 microsatellite loci.

No.	Lokus	Alel no.	Kb	Sampel
1.	mDz78B2	6	841	Gareng
		8	814	Trijoto
		11	760	Jangkarbumi
		13	720	Dewisinto
		16	646	Abiyoso
		20	388	Ponconoko
		21	375	Gareng
		38	166	Noroyono
2.	mDz3D11	1	884	Pendowo
		2	860	Ngastino
		5	836	Pancatnyono
		6	830	Anjani
		7	828	Betorokolo
		8	824	Bimo
		11	804	Pancatnyono
		17	646	Cokro
		24	564	Tirtonoko
		28	485	Rahwono
		31	455	Pendowo
		32	440	Janoko
		33	400	Tirtonoko
		36	281	Yomodipati
		38	250	Suryo

Analysis of Genetic Diversity 41 Durian Accession Using Three Microsatellite Markers

Analysis of genetic diversity aims to uncover the genetic diversity and at the same molecular characteristics of each group identified accession. Knowledge of genetic diversity is very important, because it can be used in the breeding program, especially in the selection for the assembly of new superior plant that has the desired properties. Testing genetic diversity accession durian can be done with a cluster analysis with UPGMA method (Unweighted Pair-Group With Arithme Average) using NTSYS software version 2.1 (Rohlf, 2000).

Based on cluster analysis presented in dendogram (Figure 2) it is known that all 41 accession durian formed two groups, the degree of similarity obtained coefficients ranged from 0.72 to 0.97 indicates that alll 41 accession durian does not vary much. The result showed nearly identical genetic coefficient is 0.97. Identical resemblance to approach 100% or suspected because the analysis only uses a limited locus. Microsatellite spread in the plant genome (Solihin, 2000), so it requires further analysis using microsatellite loci amount more to improve the accuracy of the observations of kinship between cultivars or varieties were observed.

The analysis uses three primary coefficient of 0.97 was found one group of cultivars of durian said their sinonime namely "*Romowijoyo* and *Abiyoso*". The greater the genetic similarity coefficient, the greater the genetic similarity. According Cahyarini *et al*. (2004) is said to be much similarity or different if the similarity coefficient of less than 0.6. This indicates that the 41 accession durian studied genetic diversity is relatively narrow. The cause of the narrow genetic diversity may be due to genetic material used in this study came from the area of origin is the same collection in situ Bawen collection. Other causes are several accession durian in this study is derived from the same parent. Narrow genetic diversity showed that all 41 accession durian is less effective for the selection of the characters that exist in the population. According Syukur *et al*. (2011) to increase the genetic diversity necessary to hybridization with other populations that have different genetic relationship with the accession tested.

Generally in dendogram with a coefficient of 0.72 was formed two large groups (A and B). The division of the cluster in large groups (A) are divided into 3 groups, where the group I, II and III respectively were 21, 11 and 5 accession. At 0.79 coefficient are group III where the locus mDz78B2 accession has 194 bp allele size becomes identifier in this group. In the group (B) there is only one group that is group (IV) consisting of accession "*Gareng, Ontorejo, Trijoto* and *Noroyono*". The existence of allele became identifier of this group is based on the locus mDz03H9 is 154 bp allele.

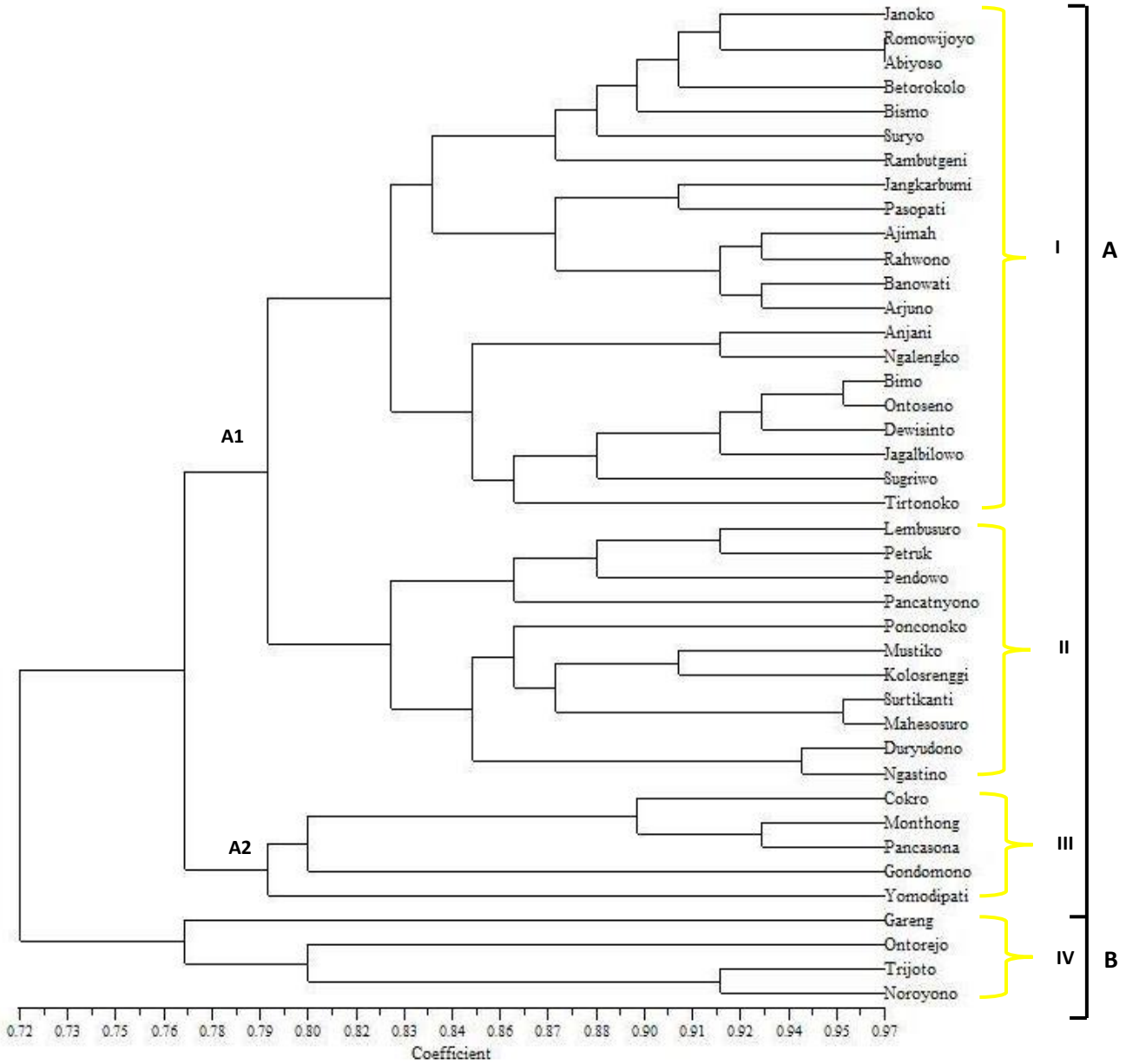


Figure 2. UPGMA clustering of 41 durio accessions in *ex situ* collection Hortimart Bawen Central Java based on three microsatellite primers.

The formation of two groups A and B are distinguished by the appearance of the size of 500-800 bp allele (locus mDz3D11) found only in group B. This means that range alleles of which is characteristic of the group B and no specific alleles were detected. While on mDz78B2 locus specific alleles detected, even appearing two alleles at

the same accession, namely on accession "*Gareng*". In group A mDz78B2 loci detected by 8 accessions that have specific alleles. Group A in the group (I) is a group that many find their specific allele, while being in the group identifier (II) the 223 bp allele at the locus mDz78B2.

CONCLUSION

The frequency of allele were mDz3D11 (1,2-12,2%), mDz03H9 (1,2-13,4%) and mDz78B2 (1,2-12,2%). Parameter Local durian diversity using microsatellite markers demonstrated high yields and is able to detect specific alleles. Phylogenetic relationship of 41 accessions of local durians are classified into two groups with the level of genetic similarity ranged 0.72-0.97.

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Improvement Nutrient-Poor Soil and Pb Level Using Soil Drive Nutrient (SDN) Method in Cropping Combination of *Zea mays* L., *Hevea brasiliensis* and L. *Paraserianthes falcataria*

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ABSTRACT

Tin mines former land required recovery efforts. It was because the content of heavy metals was quite large (one of them was Pb) in addition to nutrient- poor soil. During these improvement efforts pursued a variety of approaches like phytoremidiation. However either restored the land productivity again and could be planted no less important, because it might directly be used by the community to grow crops. Therefore in this study aimed to combine the application of phytoremidiation as an effort to cope with the heavy metal content while restoring land into productive again. Such efforts by using a combination of *Hevea brasiliensis* and *Parasianthes falcataria* L. planted with plate method or Soil Drive Nutrient (SDN) on the main crops of corn (Z. mays L.) with the help of *Glomus* sp. The results showed that a dose of 60 grams of mycorrhiza on *Hevea brasiliensis* and L. *Parasianthesfalcataria* effected on soil quality improvement and growth of corn. It characterized by decreased levels of heavy metals Pb and increases in the value of N, P, and K.

INTRODUCTION

Bangka Island was the largest island's tin producer in Indonesia which started mining tin around the 18th century (Sujitno, 2007). It has former tin mining land area reached 400,000 ha (Sitorus *et al*., 2008). In contrast to the native land, it has sand fraction more than 94%, the clay fraction was less than 3% and the content of organic C of less than 2% (Nurtjahya, 2005). In addition to the conditions of tin mined lands also contained various heavy metals which amounted to 3.040 ppm Fe, Mn of 1.9 ppm, 1.9 ppm for Cu, Pb at 6.29 ppm, 0.37 ppm Cd, Co and Cr 0.37 ppm and 1.43 ppm (Sitorus and Badri, 2005). Land conditions thereby causing tin mined land might not be used again as a productive land so that the necessary efforts to restore degraded areas through reclamation and revegetation program (Inonu, 2008).

Repair tin mined land into productive land is needed, especially in the agricultural sector. This was because the demand for agricultural products is increasing and on the other hand causes a narrowing of agricultural land occurs because of land conversion from agriculture to mining (Margarettha, 2010). One cultivated crop that necessary is maize (*Zea mays* L.). However, the problems that arisde during this time that agriculture in the former tin mine land hard done by farmers in general as a very critical condition requiring highly intensive improvement efforts (Hafizionia, 2008). Therefore, new methods are needed to improve the tin mined land to increase cultivation of corn (Z. mays L.) is the plate method (SDN). It was a method of cultivation in which we combined two infected-mycorrhizal plants which formed a roots cup and 1 main crop that was maize (Z. mays L.) that also able to be infected by mycorrhizal (Muhibbudin, 2014). Therefore this research was aim to test

effective dosage on two combination of roots cup in term of increasing maize growth that cropped in former tin land.

In this study we used a rubber tree (*Hevea brasiliensis*) and sengon (*Paraserianthes falcataria* L.) as combination of cup plant. The use of rubber trees as a crop cup 1 due to it has wide adaptability and able to live in the land of former tin mine (Inonu, 2008). In addition (Rahmawati, 2014) in a study stated that the rubber plant capable of symbiosis with mycorrhizae. Then sengon usage as 2nd crop plant caused *P. falcataria* L. was a plant that might be grown on marginal land. Moreover, according to Nusantara (2002) sengon was a plant that is capable of symbiosis with mycorrhizae

METHODS

Land-Media Preparation

On land preparation, the first step was the process of clearing land. Then set up artificial pot that was straight from the land by digging and shaping it into a square shape with a length of 1.5 m and a width of 1 m and a depth of 45 cm by 18 artificial pots. The distance between plots pot that was equal to 1 m. The artificial pot lined with plastic was quite thick on the entire surface.

Cropping Media Preparation

Planting medium that has a resembling soil texture on the former tin mine and enforced as closely as possible to the ground state of the former tin mine is used as Planting medium. The first step prepared the ground very fine sand. It was because the texture was sand (Lakitan, 2004). Then sand soil samples are inserted into each artificial pot of ± 544 kg per pot and sterilized with 1 liter of formalin 2% for each pot. After that is covered with plastic for 3 days. It is opened to the wind dried. It added with heavy metals which amounted to 1887 mg Pb and Cu at 570 mg for each pot. Organic material then added in the form of 5 kg of compost.

Mycorrhizal Inoculation and Cropping Process

This study used three types of plants, plant *H*. *brasiliensis* as the first cup, *P. falcataria* L. as a second cup of plant and plant maize (Zea mays L.) as the main crop. The third planting of these crops carried out at different times. Plants were first cultivated plant which was *H. brasiliensis*., after 2 weeks interval it planted by crops *P. falcataria* L. Furthermore, after 3 weeks of planting the main crop is growing for maize (Z. mays L.). In the process of planting the cup, each planting hole or per stands of these plants were inoculated by mycorrhizal *Glomus* sp using nominal doses that has been determined as 0 grams, 20 grams, 40 grams, 60 grams, 80 grams and 100 g. Then for the cultivation of maize (Zea mays L.) each planting hole inoculated with mycorrhizal *Glomus* sp with optimum dose to all the treatment that was 30 grams.

SDN and Main Crop Administration

Plant maintenance is done by watering the plants every day in the morning and afternoon. Thinning process of major crops is carried out 2 weeks after planting by pulling up the roots and leaves of the plants healthy.

Data Observation

Morphological observation of plant growth for *H. brasiliensis* and *P. falcataria* L. conducted every 1 week Harran and Ansori, 1993). Then plant corn (Z. mays L.) is performed every four days. The data were observed as

plant height, leaf number and stem diameter. Labratorium weight measurement process wet and dry weight as well as the observation of the percentage of mycorrhizal infection. Here is a working mechanism in the observation percentage of mycorrhizal infection is by making preparations for the semi-permanent roots first. The first working step that must be done is taken the plant roots ± 5 grams, it washed with distilled water. The washed roots stored in Formalin Acero-Alcohol (FAA) for fixed prior to the painting process. Then the roots immersed in 10% KOH and heated with an autoclave for 15-20 minutes at a temperature of 121ºC. It washed with clean water. Furthermore, the roots bleached with alkaline hydrogen peroxide, and washed again with water. After that, the roots acidified with HCl 1%. Then a solution of 1% HCL was removed and the roots soaked in a solution of trypan blue paint with a concentration of 0.05%. It heated by autoclave at a temperature of 121ºC for 15 minutes. Furthermore, the paint was removed and the roots soaked in lactoglyserol. After soaking in lactoglyserol the final step, namely the roots were prepared on glass objects up to 10 pieces of root. Then observed put under a microscope (Rossiana, 2009). The root sections observed microscopically 100- 250X magnification. The percentage of mycorrhizal inoculum infection *Glomus* sp. calculated based on the number of infected root of 10 pieces of roots were observed on each plant. Infected root characterized by vesicles or Arbuskul the root cortex and by (Rossiana, 2009) percentage of mycorrhizal inoculum infection Glomus sp.dapat calculated using the formula:% infection = number of infected roots / total Roots X 100%

Design and Data Analysis

This study used a randomized block design (RAK) with 5 different levels of treatment and one control treatment in three replications. The treatment is tested in the form of differences in dosing cup mycorrhiza in plants. Mycorrhizal dose used is 0 grams, 20 grams, 40 grams, 60 grams, 80 grams and 100 grams of the plant saucer that is pertegakan plant *Hevea brasiliensis* and *P. falcataria* L. Then for a dose of major crops of corn (Z. mays L.) which is exclusively given fixed dose of 30 grams of perlubang planting.

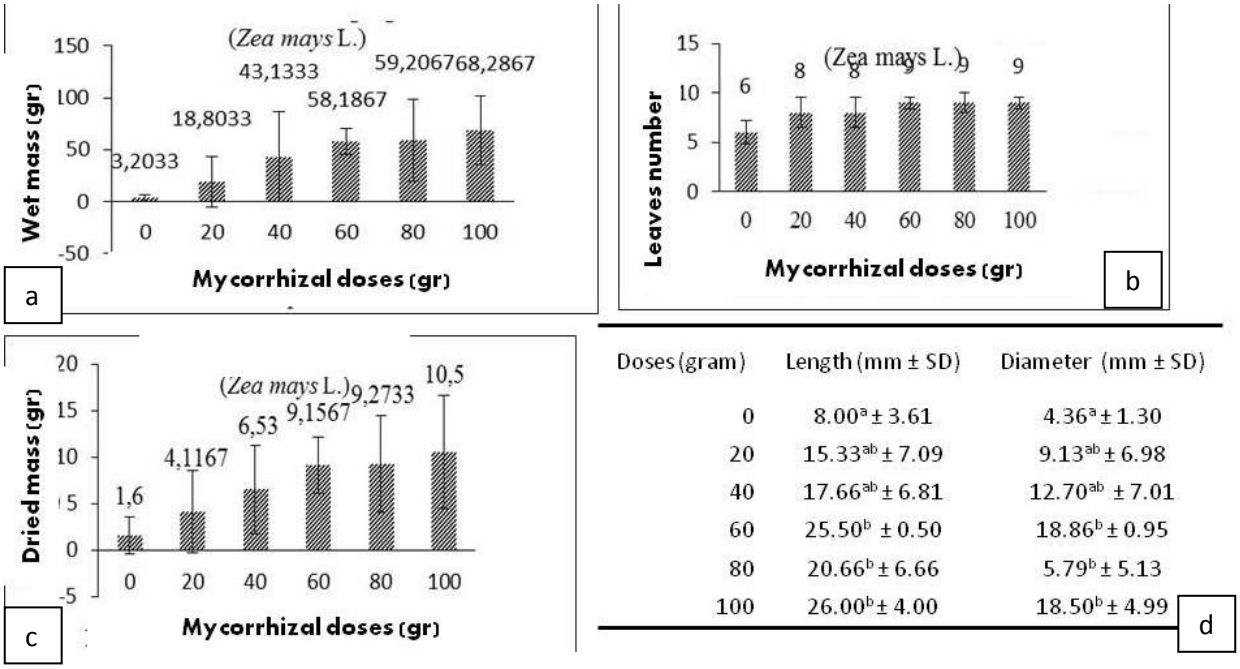
Analysis of the data in this study used one-way ANOVA with significance level (α) of 0.05 in SPSS to determine the fingerprint manifold. If the results show a significant effect, the statistical analysis will be continued using Duncan test in SPSS

RESULTS AND DSICUSSIONS

Based on the results of several parameters Annova growth of corn (Z. mays L.) can be seen that there are some parameters that differ significantly and there are some parameters did not differ significantly between treatments. In the treatment parameters tall corn plant has a significance value of 0.035 and for the treatment parameters stem diameter of corn plants have a significance value of 0.048 which means that both parameters are significantly different. Then conducted further tests such as the Duncan test and calculating the standard deviation on these parameters.

Further to Duncan test results on the diameter of the corn crop is seen that the results are the smallest diameter on corn (Z. mays L.) were treated with a dosage cup mycorrhizal 0 grams. Then there were the largest diameter on corn (Z. mays L.) that treated with a dosage cup mycorrhizae 60 grams. The corn crop is given dosage of mycorrhiza 0 gram different but not real with corn plants that are given the treatment cup 20 grams and 40 grams. Then the corn plants treated with a dosage cup 20 and 40 grams were significantly different with corn plants treated with a dosage cup 60, 80 and 100 grams. But a dose of 60, 80 and 100 grams is not significantly different from one another. The amount of trunk diameter at treatment doses of mycorrhiza 60, 80 and 100 grams showed that mycorrhiza provided an important role in improving soil and provider of nutrients in the soil for the development of stem diameter depends on the availability of nutrients in the soil, especially P which plays a role in the division and development plant cells. This is in accordance with the opinion of Lakitan (2004) which states that phosphorus is involved in the cleavage and the formation of the cells of the roots and stems of plants. Here is a picture of the appearance of the trunk diameter measurement corn (Z. mays L.) when harvesting. In

addition to the observation parameters which have significantly different results, the research also found that the observation parameters have results that are not significantly different. The parameters are not significantly different in the parameter number of leaves, fresh weight of maize (Z. mays L.) and dry weight of maize (Z. mays L.). On the parameter number of leaves have a significance value of 0.086.



Doses (gram)	Length (mm ± SD)	Diameter (mm ± SD)
0	$8.00^{a} \pm 3.61$	$4.36^{a} \pm 1.30$
20	$15.33^{ab} \pm 7.09$	$9.13^{ab} \pm 6.98$
40	$17.66^{ab} \pm 6.81$	$12.70^{ab} \pm 7.01$
60	$25.50^{b} \pm 0.50$	$18.86^{b} \pm 0.95$
80	$20.66^{b} \pm 6.66$	$5.79^{b} \pm 5.13$
100	$26.00^{b} \pm 4.00$	$18.50^{b} \pm 4.99$

d

Figure 1. Growth data based on observation for a) total wet mass, b) leaves number, c) Dried mass, d) stem length and diameter.

Based on the Fig 1 showed that the number of leaves on corn (Z. mays L.), with the effect of plant saucer 0 gram dose had the lowest number of leaves that was 6 strands. Then the dose effect of plant saucer 20 and 40 grams has a number of leaves is higher than the dose effect of plant saucer 0 grams is numbered 8. Then for the highest number of leaves found on corn (Z. mays L.) with the effect of a dose of plant saucer 60, 80 and 4100 grams is numbered 9. the results show that the higher the dose effect of mycorrhiza on plants cup, the more did the number of leaves on the plant. It is suspected high levels of nutrients available to spur the hormonal activity in the formation of the leaves. Next is the wet weight parameter of maize (Z. mays L.). In the wet weight parameter corn plant has a significance value of 0.170.

It also informed by Fig 1 that the highest plant fresh weight in plants with a dose of 100 grams and the lowest dose of 0 g. However, at doses of 60, 80 and 100 grams of the range difference is not too far away so it looks almost the same. The amount of plant fresh weight at doses of 60, 80 and 100 showed that a dose of mycorrhizae affect the growth of plants because of the presence of mycorrhizae so many nutrients that can be absorbed by plants. This is because the plant fresh weight is a reflection of the nutrient composition. The statement according to research studies (Harran and Ansori, 1993) states that the plant fresh weight is a reflection of the composition of nutrients, plant tissue by including the water content. Then other literature also states that the plant needed to form a network of some nutrients, with the addition of nutrients to the plant tends to increase the weight of the wet plant. The growth of plant organs such as roots, stems and leaves will determine the dry weight of plants.

Table 1. infection of mycorrhizal on research admistration.

Doses (gram)	Infection	
	Rubber (% ± SD)	Sengon (% ± SD)
0	36,67[a] ± 12,58	31,67[a] ± 10,41
20	78,33[b] ± 11,55	75,00[b] ± 13,23
40	88,33[ab] ± 7,64	98,33[c] ± 2,89
60	93,33[c] ± 5,77	95,00[c] ± 5
80	96,67[c] ± 5,77	100,00[c] ± 0
100	100,00[c] ± 0.00	100,00[c] ± 0

Figure 2. Roots cup formation through two cup plants.

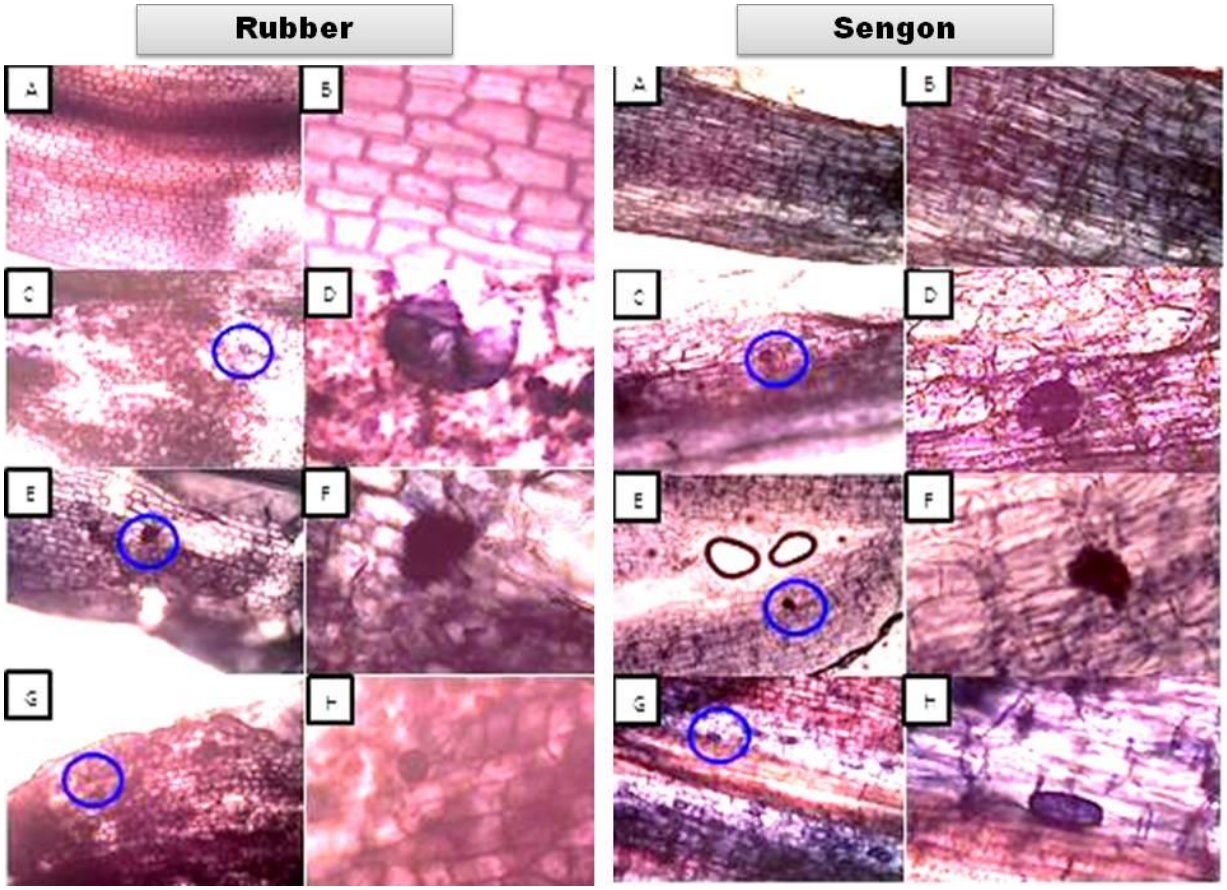


Figure 3. Mycorrhizal Infection in sengon and rubber plant

In observation of the percentage of mycorrhizal infection in rubber and sengon show significant results on tests Annova. Then these results followed by Duncan test using a 0.05 significant degree on the level of 95% indicates that the percentage of root infection was lowest for the rubber plant rubber trees are given a dose of mycorrhizal 0 grams. Then the percentage of the rubber plant infection had seen increasingly in the treatment dose of 40 grams, 20 grams, 60 grams, 80 grams and 100 grams. The percentage of a rubber plant infection at doses of 0 g looks different with a dose of 40 grams. But significantly different with a dose of 20 grams, 60 grams, 80 grams and 100 grams. Then the percentage of rubber plants infection wassignificantlydifferent at 20 gram dose, 60 grams, 80 grams and 100 grams. Then for the percentage of gum infection at a dosage of 60 grams, 80 grams and 100 grams were not significantly different. But of the three plants is seen that the percentage of infection is highest at a dose of 100 grams. 5 Furthermore, the results of Duncan test using a significant level of 0.05 at the 95% confidence level showed that the percentage of the plant's roots sengon infection was lowest for the plant roots sengon given doses of 0 g. Then the percentage of mycorrhizal infection in sengon also had seen increased with increasing dose given. Sengon percentage plant infection at doses of 0 g looks significantly different from the dose of 20 grams. Then to a dose of 40 grams, 60 grams, 80 grams and 100 grams looks significantly different from the doses 0 grams and 20 grams. But of the four doses can be seen that the percentage of infection is highest at a dose of 100 grams. It showed that mycorrhizae affect the percentage of mycorrhizal infection in the roots of plants. Plants inoculated mycorrhizal roots will have a higher percentage than plants that do not inoculated mycorrhizal (Rossiana, 2009). This condition indicates that the rubber plants and sengon able to associate with mycorrhizal Glomus sp. This is supported by the statement Setiadi (2001) that can arbuscular mycorrhizal fungi associated with almost 90% of plant species in which each type of plant can also symbiotic with one or more types of MVA. The statement also confirmed by the results of Rahmawati (2014)

which states that the rubber plant capable of symbiosis with mycorrhizae. In addition Nusantara (2002) in a study stating that the plant sengon (P. falcataria L.) is a plant that is capable of symbiosis with mycorrhizae.

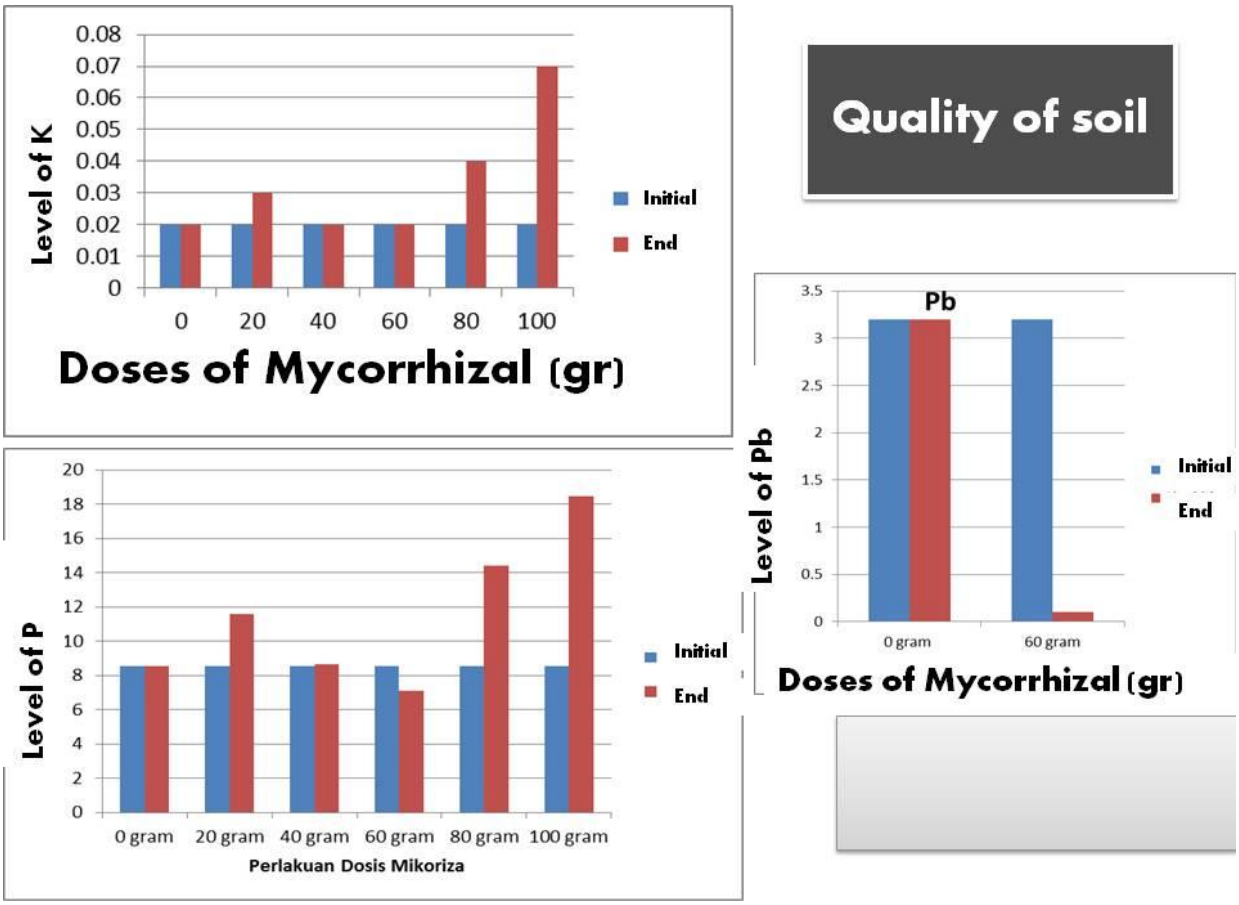


Figure 4. Plant responses in the term of P, K and Pb level changing.

Based on the Fig. 2 tangle of roots which infected by mycorrhizalshowed that there is a tangle of roots that continued to connect from one plant to another plant. At the beginning of mycorrhiza given on plant cup namely rubber and sengon. Then after the cup then infect plants will form mycorrhizal hyphae external to the plant roots cup so interwoven roots bermikoriza be a long and continued to connect. The number of interwoven roots that form is a result of the provision whereby when the plants inoculated mycorrhizal mycorrhizal then the plants will form a network of external hyphae where the external hyphae were able to improve the soil structure. This is in accordance with the opinion Pujiyanto (2001) mycorrhizal fungus hyphae through the external network can improve and stabilize the soil structure. Secretion of polysaccharide compounds, organic acids and mucus by external hyphae network able to bind primary grains into micro aggregates. "Organic binding agent" is very important in the stabilization of the micro aggregate.

Based on the Fig 4 shown in general that the P content increased in the land. The value of total soil P increased in a dose of 80 grams and 100 grams. Then the value of P at doses of 0 grams and 40 grams does not change the value of P. At a dose of 20 grams increased and decreased the value of P at 60 grams. It shows that the higher the dose effect of mycorrhiza on plant bowls, the more the value of P in the soil. It is suspected the plant bermikoriza able to increase the value of P. This is consistent with research that mycorrhiza-infected plants are able to absorb elements of P is higher than uninfected plants (Suhendry *et al*., 1996).

The mechanism of mycorrhiza in K nutrient increment used absorptionextention of nutrients by external mycelium. The presence of mycorrhizal symbiosis with plant roots not only helps in the absorption of nutrients P, but is able to increase the absorption of other nutrients, both micro and macro.

Heavy metal test results showed that the levels of heavy metals Pb pnurunan on the ground after being tested AAS. At the dosage of mycorrhiza 0 soil containing heavy metals Pb of 3.2 ppm. While on treatment dose of 60 grams of mycorrhiza contained heavy metals Pb of 0.01 ppm. This indicated that mycorrhiza can lower levels of heavy metals in the soil Pb. This statement is in accordance with the literature (Rossiana, 2009) which states that bermikoriza plant roots can play a role in reclaiming lands that are contaminated by heavy metals

CONCLUSSIONS

The conclusion in this study was a dose of mycorrhiza on plant *Hevea brasiliensis* and *Paraserianthes falcataria* L. affected on soil quality improvement and growth of corn plants on the parameters plant height and diameter. There was also a significant effect on the percentage of mycorrhizal infection in plants *H. brasiliensis* and *P. falcataria* L. gave the same result, or not significantly different with maize growth and the percentage of root infection in plants. So that the most effective dose is selected dose of 60 grams of mycorrhizal, In general, there was an increase in the value of N, P, K and at a dose of 100 grams mycorrhizal there was an increase the value of N, P, and K and influential in the improvement of soil quality.

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Effect of Addition of Kepok Banana (*Musa Paradisiaca Linn*) Peel Flour as A Stabilizer to Chemical And Organoleptic Characteristic of Ice Cream

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ABSTRACT

Pectin characteristic in kepok banana peel is used to be stabilizer in making ice cream process. Aim of research was determined effect of amount addition of kepok banana flour precisely so it could produce ice cream with best of chemistry and organoleptic characteristic. The research was arranged by non factorial Random Complete Block Design (RCBD) with four replications. The treatment was given on each replication was the amount of kepok banana peel that consisted of six different levels, they were 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, and 0.6% (w/v). As reference, researcher was added gelatin 0.5% as stabilizer which it was used to analyze organoleptic property. The observations of research were organoleptic property, overrun, emulsion stability and melting time. Best result of observations was analyzed about proximate property of ice cream. The amount of kepok banana peel was 0.1% (w/v) that showed results of organoleptic property: aroma score was 3.20 (bit of banana); flavor score was 3.85 (sweet); color score was 3.66 (brown); texture score was 4.00 (soft); and acceptable panelist to product was 3.48 (bit of like). Results of proximate analyze were: water content was 63.48%; protein content was 1.37%; fat content was 2.20%; ash content was 1.13%; fiber content was 1.56%; and carbohydrate content by different was 30.26%.

Keywords: Kepok banana peel, stabilizer, ice cream.

INTRODUCTION

Banana is one of the horticultural commodities which have potential and high economic value both domestically and for export. In Indonesia, bananas occupied the first place among other types of fruits, both in terms of distribution, area planted, and in terms of production. Indonesia total banana production in 2013 was 5.359.126 tons and Lampung accounted for 678.492 tonnes or 12.66 % of the national banana production (BPS, 2014). When all the bananas are better utilized as direct consumption of food or processed products, the problem that arises is the waste generated from the utilization of bananas.

Kepok banana (*Musa Paradisiaca L.*) is often used as a raw material for making banana chips in Lampung . Waste from kepok banana chips industry is a potential banana peel has a sale value if used as a product. Kepok potential banana peel to produce pectin compound .Ahda and Berry (2008) stated content of pectin in banana peelkepok ranged between 10.10 % -11.93 % . Pectin is widely used as a functional component in food because of their ability to form and stabilize aqueous gel emulsion (May, 1990 in Hariyati, 2006).

The nature of the pectin stabilizer kepok banana peel can also be used as a stabilizer in the process of making ice cream. But until now, no study related to the use of flour banana peel kepok as a stabilizer and the amount of flour banana peel kepok that can be added to the ice cream making process so as to produce the best chemical and organoleptic properties. For that, we need to do research on The Use of Peel Flour Kepok Banana (*Musa paradisiaca Linn*) As a stabilizer of the Chemical Properties and Appearance Ice Cream . The research is expected to determine the amount of flour banana peel kepok use in the manufacture of ice cream to produce chemical and organoleptic properties of ice cream.

MATERIALS AND METHOD

Materials and Tools

Materials used in the study is kepok banana peel obtained from Central Banana Chips Industry Bandar Lampung, full cream milk powder (Indomilk), skim milk powder (Indomilk), sugar, water, and egg yolks. Chemicals for analysis is hexan, concentrated H_2SO_4, H_2SO_4 1.25 %, 1.25% NaOH; 50% ; 1 N, 0,02N HCl, H_2BO_2 , $Na_2S_2O_3$, citric acid, $CaCl_2$, distilled, indicators PP, and alcohol. Tools used in the study include knives, scales, stove, mixer, freezer, box freezer, pans, spoons, mixer stick, basins, thermometers, refrigerators, autoclaves, petri dishes, bottles, soxhlet, desiccator, furnace, porcelain dish, buchner funnel, measuring cups, oven, plate metal, kjeldahl flask, erlenmeyer, filter paper, pipette, analytical balance, glass tools and a set of tools supporting organoleptic test.

Research Method

The research was arranged by non factorial Random Complete Block Design (RCBD) with four replications. The treatment was given on each replication was the amount of kepok banana peel that consisted of six different levels, they were 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, and 0.6% (w/v). As reference, researcher was added gelatin 0.5% as stabilizer which it was used to analyze organoleptic property. The observations of research were organoleptic property, overrun, emulsion stability and melting time. Best result of observations was analyzed about proximate property of ice cream. All data were obtained unless the melting time parameters tested in common manifold using bartlet test and tuckey test. Data were analyzed by analysis of variance to get the error variance estimators. The data analysis followed using HSD test at 5% level. While the melting time parameter data analyzed descriptively.

The Implementation of Research

Begins with a banana peel flour kepok (modified from Rois, 2012) where the banana peel kepok 1 kg washed with clean water and then drained. Then soaked in warm water for 10 minutes at a temperature of 70°C. After it was dried with dried under the sun until dry cured banana peel. Kepok banana peel dry milled using a disc mill and milled sieved to 40 mesh size. The result kepok flour banana peel as much as 300 grams. Then proceed with the making of ice cream (modified from Widyanti, 2002). Raw materials such as water (70.9%), milk cream (10%), skim milk (7%), and sugar (12%) are mixed until dissolved. Selanutnya pasteurized at a temperature of 63°C for 30 minutes. During the pasteurization process was added treatment flour banana peel kepok by the number of additions of 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, and 0.6% (w/v) and gelatin 0.5% as a reference in examining the organoleptic properties of the ice cream is then added egg yolk as much as 2 points. Once it is done using the material homogenizing mixer for 1 hour. The batter is homogeneous given aging treatment in the freezer for 4 hours. Then the dough is stored in a deep freezer until frozen into ice cream.

Observation

The observations of research were organoleptic property(Watts et al., 1989), overrun(Marshall dan Arbuckle, 2000), emulsion stability (AOAC, 2005) and melting time(Roland et al., 1999). Best result of observations was analyzed about proximate property of ice cream such asthe water content (AOAC, 1984), fat content (Sudarmadji, 1984), protein content(Sudarmadji, 1984), ash content (AOAC, 1984), crude fiber content (Sudarmadji, 1984), and by different carbohydrate (Winarno, 1992).

RESULTS AND STUDY

Organoleptic Characteristic

1. Aroma

Results of analysis of variance showed the addition of banana peel flour has no effect on aroma ice cream. Results of aroma of ice cream has an average of 3.07.

Table 1. Score of ice cream aroma with the addition of banana peel flour.

Treatments	Score
C1= The addition of banana peel flour 0.1%	3.20
C2= The addition of banana peel flour 0.2%	3.28
C3= The addition of banana peel flour 0.3%	2.80
C4= The addition of banana peel flour 0.4%	3.05
C5= The addition of banana peel flour 0.5%	3.03
C6= The addition of banana peel flour 0.5%	3.08

Note:
1 :Very typical of banana
2 : Typical of banana
3 : Bit typical of banana
4 : Not typical of banana
5 : Not very typical of banana

The use concentration of banana peel flour does not affect the aroma of ice cream. Aroma ice cream in general is the aroma and flavor of milk sugar (Rachmawati and Handajani, 2011). Extra banana peel flour aroma typical decrease in milk in ice cream . In addition, the starch hydrocolloids can slightly reduce the intensity of the odor, flavor and aroma of a solution (Trenggono et al., 1989). Aroma arising from banana peels due to the volatile compounds contained in a banana peel evaporates. According to research Noorohmi (2010) yield of banana peel extract obtained from the remaserasi of 13.305 %, in the form of dark brown liquid with a banana aroma less sweet and more smelling sap. Types of volatile compounds contained in a yellow kepok banana peel identified on the basis of research Noorohmi (2010) is pentadekanoat acid and acid –9,12 oktadekadienoat.

2. Flavor

Results of analysis of variance showed the addition of banana peel flour on the ice cream affect the flavor of ice cream . Results organoleptic ice cream flavors ranging between 2.71 - 3.85 (slightly sweet - sweet). Further test results HSD at the level of 5 % of ice cream flavors are presented in the following table.

Further test results HSD 5% indicates that the treatment of C1 (0.1%) in contrast to the treatment C2 (0.2%), C3 (0.3%), C4 (0.4%), C5 (0.5%) and C6 (0.6%). Scores on the treatment of C1 is 3.85, which means flavor in ice cream is sweet. Scores on the treatment of C2, C3, C4, C5, and C6 respectively - are respectively 3.09, 3.18, 2.74, 2.86, and 2.71. Flavor between the treatment of C2, C3, C4, C5, and C6 is no different with scores ranging from 2.71 - 3:18. Treatment C2, C3, C4, C5 and C4 have a bit of sweet flavor. Differences in sweetness caused by the use of flour peel in the process of making ice cream. This is because the flour banana peel contains pectin compounds that give a bitter effect. According Hartoyo (2003) in tea include caffeine content (2-3%), theobromine, theofilin, bumin adenine, polyphenols, fiber, pectin, and catechins that can cause a bitter and astringent flavor. In addition to causing a bitter flavor, adding flour banana peel causes sweetness decreases. According Trenggono et al. (1989), a decrease due to the nature of the sweet flavor of starch hydrocolloids capable of reducing the intensity of the flavor of a solution. A decrease in the intensity of flavor due to the nature of the displacement, the rate of diffusion of molecules that bring nature to the sense organ of flavor slowly. Hydrocolloid coating on the tongue serves as a diffusion barrier.

Table 2. Test HSD 5 % up on the flavor of ice cream with the addition of banana peel flour.

Treatments	Score
C1= The addition of banana peel flour 0.1%	3.85 [a]
C2= The addition of banana peel flour 0.2%	3.09 [b]
C3= The addition of banana peel flour 0.3%	3.18 [b]
C4= The addition of banana peel flour 0.4%	2.74 [b]
C5= The addition of banana peel flour 0.5%	2.86 [b]
C6= The addition of banana peel flour 0.6%	2.71 [b]
HSD (0.05) = 0.49	

Note: Scores followed by the same letter do not differ on the test showed HSD Test level of 5%
Note:
1 : Not very sweet
2 : Not sweet
3 : Bit of sweet
4 : Sweet
5 : Very sweet

According to Winarno (1992), that there are several factors that influence consumer acceptance of flavor, among other chemicals, temperature, concentration and interaction with other flavor components. Products that have a bad flavor will not be accepted by consumers despite the color, aroma, and texture good. Therefore, flavor is one of the important factors in the consumer's decision to accept or reject a product.

3. Color

Results of analysis of variance showed the addition of banana peel flour on the ice cream to give effect to the color of the ice cream. Results of organoleptic color ice cream range in 3.66 – 4.33 namely between bit of brown to brown. Further test results HSD level of 5% are presented in the following table.

Table 3. Test HSD 5 % up on the color of ice cream with the addition of banana peel flour.

Treatments	Score
C1= The addition of banana peel flour 0.1%	3.66 [b]
C2= The addition of banana peel flour 0.2%	3.85 [ab]
C3= The addition of banana peel flour 0.3%	3.99 [ab]
C4= The addition of banana peel flour 0.4%	3.78 [ab]
C5= The addition of banana peel flour 0.5%	4.01 [ab]
C6= The addition of banana peel flour 0.6%	4.33 [a]
HSD (0.05) = 0.61	

Note: Scores followed by the same letter do not differ on the test showed HSD Test level of 5%
Note:
1 : White Yellowish
2 : White Brownish
3 :Bitof brown
4 : Brown
5 : Very brown

Further test results HSD at 5% level treatment C1 (0.1%) the results are different from the treatment of C6 (0.6%) and did not differ by treatment C2 (0.2%), C3 (0.3%), C4 (0.4%) and C5 (0.5%). Scores of C1 is 3.66 which is a bit of brown and a score of C6 is 4.33 which is brown. C6 treatment did not differ by treatment with C2, C3, C4, and C5, but in contrast with the treatment of C1. Extra banana peel flour on the ice cream affect the color of ice cream. Brown color differences occur due to the amount of use kepok banana peel flour on the ice cream. The higher the amount of banana peel flour written kepok the more brown color ice cream also produced. This is because the banana peel flour contains phenolic compounds that undergo the browning reaction (browning) due to exposure to oxygen and heat that arise when direct drying.

According Suparmi and Harka (2012), phenol content on banana peels are 0.9 - 3g / 100g. Browning occurs due to oxidation with air to form browning reaction by the effect of enzymes found in these food ingredients (enzymatic browning). Browning because the enzyme is a reaction between oxygen and a phenol compound catalyzed by polyphenol oxidase. The formation of brown color on a banana peel is triggered by an oxidation reaction catalyzed by the enzyme phenol oxidase or polyphenol oxidase. The enzyme can catalyze the oxidation of phenol into quinone compound and then dipolimerasi be melaniadin brown pigment (Winarno, 2001).

Color is one of the factors that influence consumer acceptance. According to Arbuckle (2000), color ice cream should be interesting and fun consumers, uniform, and can represent flavors are added.

4. Texture

Results of analysis of variance showed that the addition of banana peel flour on the ice cream produced an effect on the texture.Results of organoleptic texture of the ice cream is between 2.83 – 4.00 which is bit of hard until soft. HSD test results 5% level are presented in the following table.

Table 4. Test HSD 5 % up on the texture of ice cream with the addition of banana peel flour.

Treatments	Score
C1= The addition of banana peel flour 0.1%	4.00 [a]
C2= The addition of banana peel flour 0.2%	3.38 [b]
C3= The addition of banana peel flour 0.3%	3.13 [c]
C4= The addition of banana peel flour 0.4%	3.08 [cd]
C5= The addition of banana peel flour 0.5%	2.93 [d]
C6= The addition of banana peel flour 0.6%	2.83 [d]
HSD (0.05) = 0.22	

Note: Scores followed by the same letter do not differ on the test showed HSD Test level of 5%
Note:
1 : Very hard
2 : Hard
3 : Bit of soft
4 : Soft
5 : Very soft

Further results test HSD at the level of 5% that do, treatment C3 (0.3%) is different from the treatment of C1 (0.1%), C2 (0.2%), C5 (0.5%) and C6 (0.6%), but not different treatment C4 (0.4%). Scores for the treatment of C3 is 3.13 which is bit of soft, while the score for treatment of C1 is 4 (soft), C2 is 3:38 (bit of soft), C5 is 2.93 (bit of soft), and C6 is 2.83 (bit of soft). Treatment C5 and C6 was not different between treatments, but in contrast with the treatment of C1, C2, C3. Treatment is different with each treatment C1 and C2 were different from each treatments. The use of flour banana peel affect the texture of ice cream produced. Texture difference occurs because the amount of use kepok banana peel flour on the ice cream.

Banana peel flour is dried nonfat ingredients that cause differences in physical characteristics, especially the effect on the texture of ice cream. According to research Elisabeth et al. (2007), the use of skim milk with sweet potato steamed nonfat dry ingredients seem to increase the thickness (viscosity) ICM (Ice Cream Mix), further limiting the mobility of water molecules because the space between the particles in the ICM becomes increasingly narrow. The narrowness of the space between particles causes the incoming air into the ICM during the agitation is getting a bit so that the value of the resulting lower overrun. Overrun is too low can cause the ice cream frozen into a product that is too hard and soft as pudding, while the overrun is too high causing too soft ice cream, fast melt, and has a bland flavor (Suprayitno et al., 2001). This statement is also corroborated by Syafutri (2012), the use of cucumber pulp into the dough ice cream will increase the viscosity of the ice cream, the air will be difficult to enter into the dough, causing overrun decline. Decreasing overrun cause the ice cream to be loud and mushy.

5. Acceptable of Panelist to Product

The results of the analysis of variance showed that the addition of banana peel flour on the ice cream affect the overall acceptance of the ice cream. Results organoleptic overall acceptance of the ice cream is between the value of 2.45 to 3.48 that is not like until bit of like. HSD test results 5% level are presented in the following table.

Table 5. Test HSD 5 % up on the overall acceptance of ice cream with the addition of banana peel flour.

Treatments	Score
C1= The addition of banana peel flour0.1%	3.48 [a]
C2= The addition of banana peel flour 0.2%	2.74 [b]
C3= The addition of banana peel flour 0.3%	2.95 [b]
C4= The addition of banana peel flour 0.4%	2.80 [b]
C5= The addition of banana peel flour 0.5%	2.65 [b]
C6= The addition of banana peel flour 0.6%	2.45 [b]
HSD (0.05) = 0.51	

Note: Scores followed by the same letter do not differ on the test showed HSD Test level of 5%
Note:
1 : Very dislike
2 : Dislike
3 : Bit of like
4 : Like
5 : Very like

Further test results HSD 5% indicates that the treatment of C1 (0.1%) in contrast to the treatment C2 (0.2%), C3 (0.3%), C4 (0.4%), C5 (0.5%) and C6 (0.6%). Scores on C1 treatment is 3.48, which means the overall acceptance of the ice cream is a bit like. Scores on the treatment of C2, C3, C4, C5, and C6 respectively – are respectively 2.74, 2.95, 2.80, 2.65, and 2.45. Overall acceptance among treatments C2, C3, C4, C5, and C6 is no different with scores ranging from 2:45 – 2.95.

According to Arbuckle (1986), color ice cream should be interesting and fun consumers, uniform, and can represent added flavor is one of the factors that influence consumer acceptance. This is supported by the statement of Francis (2003) in Neilsen (2003) that color is an important factor to the reception because if the product does not appeal then the product will be less attractive to consumers, so consumers will reject these products without regard to other nutritional value. However, not only in terms of color, the other factors must also be considered. A panelist differences occur due to the amount of use kepok banana peel flour on ice cream and this has an impact on the ratings of other organoleptic parameters that affect the level of preference panelists.

Melting Time

The results of observations of the melting time of ice cream with the addition of banana peel flour kepok presented in Fig. 1.

Melting time is the time required melting ice cream to melt perfectly as early dough at room temperature. Good ice cream is ice cream that is resistant to thaw at room temperature and in a certain time. According Padaga and Savitri (2005), which quickly melted ice cream is less preferred because the ice cream will soon melt at room temperature. However, the ice cream melting high time is also not preferred by consumers because of the shape of ice cream that does not change gives the impression too many solids are used.

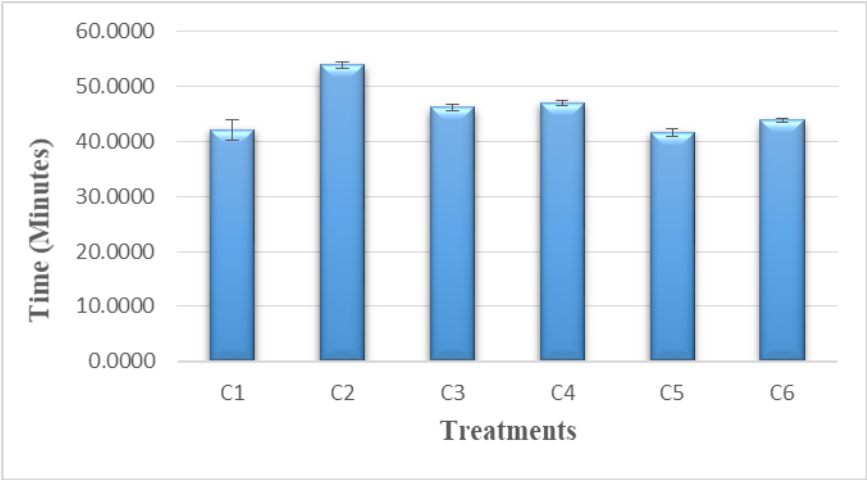

Figure 1. Melting Time of Ice Cream (Primary Data, 2016).

Based on the testing to each treatment, the time of melting is at a time over 40 minutes. According Susilorini and Savitri (2006) the time of melting ice cream was good is between 15-20 minutes. Too long melt time indicates the amount of solids contained in the ice cream (Padaga and Savitri, 2005). Extra flour banana peel is able to bind the dough ice particles in the ice cream so that the dough more viscous and the narrowing space between the particles. The narrowness of the space between particles causes the incoming air into the ice cream during agitation is getting a bit so that the value of the resulting lower overrun. Overrun value is low causing the ice cream freeze into products that are too hard so that it has a high melting time.

High melting time is at C2 treatment but after treatment C2 (C3, C4, C5, and C6) melting time decreased. This indicates pectin contained in the banana peel flour can work well. Stabilizer mechanism in maintaining the organoleptic properties of the ice cream is spread globules of fat throughout the dough so as to prevent clustering of globules of fat, as well as the stabilizer can bind the water to the dough, thereby reducing the formation of crystals of ice cream during storage. All the stabilizer increases the viscosity of non-frozen portion of the molecule to inhibit migration of crystal nuclei and consequently the crystal size is limited, so the ice cream produced tended to be soft (Goff, 2000). Means the amount of stabilizer used in ice cream, the ice cream also has a softer texture and melt velocity was decreased. However, the use of excessive stabilizer is not desired. According Padaga and Savitri (2005), the slow melting undesirable because it reflects that there are excessive stabilizer.

Further results test HSD at the level of 5% that is done, the treatment did not differ between the treatment C1 (0.1%), C5 (0.5%) and C6 (0.6%), but the three treatments are different from the treatment of C2 (0.2%), C3 (0.3%) and C4 (0.4%). Value for treatment C1, C5, and C6 are respectively 5.76%, 5.86% and 5.83%, while the value for the treatment of C2, C3, and C4 respectively is 6.26%, 6.35%, and 6.23%. The use of flour banana peel affect overrun ice cream produced. Overrun difference occurs because the amount of use kepok banana peel flour on the ice cream.

Overrun

Results of the analysis of variance showed that the addition of banana peel flour on the ice cream affect the overrun ice cream . The test results overrun ice cream ranged from 5.76 value – 6.35%. HSD test results 5% level are presented in the following table.

Table 6. Test HSD 5 % up on the overrun with the addition of banana peel flour.

Treatments	Score
C1= The addition of banana peel flour 0.1%	5.76 [b]
C2= The addition of banana peel flour 0.2%	6.26 [a]
C3= The addition of banana peel flour 0.3%	6.35 [a]
C4= The addition of banana peel flour 0.4%	6.23 [a]
C5= The addition of banana peel flour 0.5%	5.86 [b]
C6= The addition of banana peel flour 0.6%	5.83 [b]
HSD (0.05) = 0.13	

Note: Scores followed by the same letter do not differ on the test showed HSD Test level of 5%

Overrun shows the additional volume of dough ice cream because of the air trapped in the ice cream mixture due to the agitation. Overrun affect the texture and density that determine the quality of the ice cream. The higher overrun the lower the solids in ice cream products, as well as the faster melting.

Based on testing to each treatment, overrun values obtained under the national standards established by the National of Standards Indonesia. Overrun value of the test results in each treatment ranged between 5.76 - 6.35% and this result is very low compared to SNI NO. 01-3713-1995 (BSN, 1995) ranged from 70-80% (industrial scale) and 30-50% (household). According Suprayitno et al. (2001), low overrun in ice cream because at least the air trapped in the ice cream during the agitation. Extra banana peel flour solids in the dough increases the greater the more viscous so the dough. This is because the fibers have the ability to bind water in the dough ice cream. Heightened use of flour banana peel, the more the amount of bound water that causes the water content decreases so the more viscous dough ice cream and ice cream melting time is getting longer (Syafutri, 2012). According Yosephine et al (2012), the fiber content on a banana peel is 50.3g / 100g. The presence of pectin in banana peel flour also affects the viscosity of dough ice cream. This is because the starch have functional properties that thicken and form a gel (Trenggono et al., 1989).

Emulsion Stability

Results of the analysis of variance showed that the addition of banana peel flour on the ice cream emulsion affects the stability of the ice cream . The results of the emulsion stability test ice cream ranging between 60.64 - 68.95 %. HSD test results 5% level are presented in the following table.

Table 7. Test HSD 5 % up on the emulsion stabilitywith the addition of banana peel flour.

Treatments	Score
C1= The addition of banana peel flour0.1%	60.64 [e]
C2= The addition of banana peel flour 0.2%	62.52 [d]
C3= The addition of banana peel flour 0.3%	63.73 [c]
C4= The addition of banana peel flour 0.4%	65.50 [b]
C5= The addition of banana peel flour 0.5%	68.56 [a]
C6= The addition of banana peel flour 0.6%	68.95 [a]
HSD (0.05) = 0.77	

Note: Scores followed by the same letter do not differ on the test showed HSD Test level of 5%

Further test results HSD at 5% that do, treatment C1 (0.1%) in contrast to all treatments. C2 treatment was different with all treatments. C3 treatment in contrast to all treatments. C4 treatment was different with all treatments. The treatment did not differ between C5 and C6, but both of those treatments is different from all other treatments. Values treatment emulsion stability C1, C2, C3, C4, C5, and C6 are 60.64%, 62.52%, 63.73%, 65.50%, 68.56% and 68.95%. The use of banana peel flour affect the stability of the emulsion produced ice cream. The color difference occurs because the amount of use kepok banana peel flouron the ice cream. The higher the amount of banana peelflourwrittenkepok higher the stability of the emulsion produced ice cream.

Ice cream emulsion stability indicates the durability of dough ice cream on the separation of milk protein and milk fat. Unstable emulsion causes the proteins would clump and settle in it, causing a separation between protein and fat (Arbuckle, 1986). According Bodyfelt et al. (1988), emulsion stability is usually indicated by two circumstances, namely the process of forming cream and phase separation.

Based on the test results for each treatment showed increased emulsion stability value by heightening the concentration of banana peelflour. According to Arbuckle (1986) that the stability of the emulsion is affected by the type and amount of stabilizer, the size and uniformity of the fat globules, and the viscosity of the dough. Smaller and more uniform the fat globules higher emulsion stability. Therefore, the addition of banana peel flourcontains pectin as a stabilizer and a perfect homogenization process the dough will cause the dough becomes thick and has a size of fat globules so small that the resulting emulsion stability is high.

Selection of Best Treatment

Determining the best treatment based on the results of the organoleptic (aroma, flavor, color, texture and overall acceptance), test of melting time, overrun test, and test of the emulsionstability. Summary data of the selection of the best treatment can be seen in Table 9.

Table 9. Summary data of the selection of the best treatment.

Parameter	Perlakuan					
	C1	C2	C3	C4	C5	C6
Aroma	3.20	3.28	2.80	3.05	3.03	3.08
Rasa	3.85^{a}	3.09^{b}	3.18^{b}	2.74^{b}	2.86^{b}	2.71^{b}
Warna	3.66^{b}	3.85^{ab}	3.99^{ab}	3.78^{ab}	4.01^{ab}	4.33^{a}
Tekstur	3.18^{ab}	2.66^{b}	3.58^{a}	3.33^{ab}	2.99^{ab}	2.94^{b}
PenerimaanKeseluruhan	3.48^{a}	2.74^{b}	2.95^{b}	2.80^{b}	2.65^{b}	2.45^{b}
KecepatanMeleleh	42.13^{c}	53.95^{a}	46.28^{b}	47.03^{b}	42.18^{c}	43.88^{c}
Overrun	5.76^{b}	6.26^{a}	6.35^{a}	6.23^{a}	5.86^{b}	5.83^{b}
StabilitasEmulsi	60.64^{e}	62.52^{d}	63.73^{c}	65.50^{b}	68.56^{a}	68.95^{a}

Note:
C1 :Banana Peel Flour 0.1%
C2 :Banana Peel Flour 0.2%
C3 :Banana Peel Flour 0.3%
C4 :Banana Peel Flour 0.4%
C5 :Banana Peel Flour 0.5%
C6 :Banana Peel Flour 0.6%

Making the best treatment viewed from two aspects: organoleptic (aroma, flavor, color, texture, and overall acceptance) and aspects of laboratory test (melting time, overrun, and the emulsion stability). Determining the best treatment of organoleptic and laboratory testing using the method of notation star is by

weighting the letters using the number of stars. Stars awarded to the best letters are categorized based on the parameters or the closest existing standards as well as the letter did not differ with the best parameters. The result is a C1 treatment has the greatest weight.

The best treatment is obtained from the research is the treatment of C1 for product research yet to be commercialized so that aspect of laboratory tests need to be developed further to obtain results in accordance with the Indonesian National Standard ice cream products.

Proximate Analysis

Proximate analysis was conducted to determine the nutrients contained in the ice cream is by adding banana peel flour as much as 0.1% (25 grams). Proximate analysis was conducted on the water content, protein content, fat content, ash content, fiber content and carbohydrates by different. Proximate analysis results can be seen in Table 10.

Table 10. Proximate analysis of ice cream with the addition of banana peel flour 0.1%.

Parameter	Value (%)
Water content	63.48
Protein content	1.37
Fat content	2.20
Ash content	1.13
Fiber content	1.56
Carbohydrate by different	30.26

In Table 10, the nutrient content in the formulation of C1, namely ice cream added banana peel flour as much as 0.1% have a water content of 63.48%, the protein content of 1.37%, fat content of 2.20%, ash content of 1.13%, fiber content of 1.56% , and carbohydrateby different of 30.26%.

Table 11. Terms of nutritional quality ice cream.

No.	Test Criteria	Unit	Terms
1.	Fat	% w/w	Minimum 5.0
2.	Sugar calculated as sucrose	% w/w	Minimum 8.0
3.	Protein	% w/w	Minimum 2.7

Source: BSN-SNI 01-3713-1995

When viewed from the requirements of the nutritional content of ice cream on the table 11, the nutrient content in ice cream with the addition of banana peelflouras much as 0.1 % in protein and fat that not meet the requirements of SNI . This is because the use of materials that are a source of fat and protein that is full-cream milk and skim milk are not many and the addition of banana peel flour did not affect the increase in fat content and protein content due to both the inside of a kepok banana peel few. Besides the formation of the fiber in the banana peel flour written kepok can bind fat (Tensiska, 2008) so it is not counted in the analysis of fat.

CONCLUSION

The conclusion of the research is the addition of kepokbanana peel flourtreatment(C1) of 0.1% effect on the results of the ice cream with the organoleptic properties :aroma score was 3.20 (bit of banana); flavor score was 3.85 (sweet); color score was 3.66 (brown); texture score was 4.00 (soft); and acceptable panelist to

product was 3.48 (bit of like). Results of proximate analyze were: water content was 63.48%; protein content was 1.37%; fat content was 2.20%; ash content was 1.13%; fiber content was 1.56%; and carbohydrate content by different was 30.26%.

SUGGESTION

It is advisable to do the filtering dregs kepokbanana peel flourinsoluble after homogenization process to increase the overall acceptance of the panelists on the organoleptic properties of ice cream, adding sugar to the right to cover the bitter taste of ice cream arising due to the addition of kepokbananapeelflour, and perform replacement material sources of fat and protein as a raw material for making ice cream or the addition of the source material fats and more proteins to produce quality ice cream in accordance with the SNI NO . 01-3713-1995.

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Manufacture of Briquette Coconut Shell's Charcoal and Mixed Plastic and Rubber's Waste of Kupang Jabon's Landfill Sidoarjo Regency using Tapioca Adhesive as Refuse Derived Fuel (Rdf) Products

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ABSTRACT

This research aims to determine the results of the proxymate value (quality of moisture, volatile matter, ash and calorific content); best of total value and treatment from all charcoal briquette samples as RDF product; value of flame of fire, endurance and stability from the best treatment; and compared the charcoal briquette's proxymate analysis value (quality of moisture, volatile matter, ash and calorific content) as RDF product from the best treatment with SNI 01-6235-2000 of wood charcoal briquettes. The Method used is ASTM D 3173-03, ASTM D 3175-02, ASTM D 3174-02 dan ASTM D 2015-96; then given the score test the best of category total value. The best treatment is selected from the best of category total value, then used for testing flame of fire; endurance and stability. Water content; volatile matter; ash content and calorific content obtained 5.267%-7.067%; 9.367%-13.233%; 5.333%-7.967% and 5058.9 cal/g-5336.4 cal/g. Best of value obtained 68,25. The best treatment is briquette with compositions 90 g consists of 2.5% plastics; 2.5% rubbers; and 95% *coconut shell's charcoal* has screened at sieve 10 mesh. Test of flame of fire; endurance; and stability of high and diameters each obtained 111,17 minutes; 8,21%; 2,15%. The proxymate value (quality of moisture, volatile matter, ash and calorific content) by the best of treatment is 5,23%; 9,80%; 5,40% dan 5333,30cal/g accordance with SNI 01-6235-2000 of wood charcoal briquettes.

Keywords: Charcoal briquette, endurance, proxymate analysis *refuse derived fuel*, stability.

INTRODUCTION

The fuel crisis of the petroleum reserves, natural gas, and coal as long as the main energy source of fossil fuels dwindling numbers. The use of renewable energy become an important option (Prawiroadmodjo and Armando, 2005). Other resources that can be utilized as a renewable energy can be derived from natural resources and the rest of the ingredients that have been used (garbage) (Hambali et al., 2007). One example of Indonesia's natural wealth that can be used as renewable energy is utilizing coconut shell with charred, since it can generate heat of 6500-7600 kcal / kg (Gultom, 2008). In addition, the utilization of the residue will be (garbage) in the landfill can be a product and contribute to renewable energy (Ruslinda et al., 2012). Mechanical utilization of municipal waste collected in the landfill as a fuel called Refuse Derived Fuel (RDF) (Alter, 1987). In general, RDF is composed of plastic and other materials, such as rubber and wood (Chiemchaisri et al., 2010). One landfill known plastic and rubber components have potential as a raw material TPA Kupang RDF is Jabon, Kab. Sidoarjo. Plastic and rubber KupangJabon landfill can generate heat of 10.220 kcal / kg and 4,517 kcal / kg (Solomon, 2014). Therefore, in order to available renewable energy and efforts to reduce the rate of waste generation inorganic landfill KupangJabon so that the operational lifetime is longer, then made briquettes coconut shell charcoal and mixed trash types of plastics and rubber as product RDF. The purpose of this study was to obtain the composition and characteristics of the charcoal briquettes as a product of the best RDF getting the best total value category is based on the suitability of the parameters of

water content; volatile matter; ash and calorific referring to the Indonesian National Standard (SNI) 01-6235-2000 of wood charcoal briquettes and obtained the value of the flame; resilience and stability of the briquettes are.

MATERIAL AND METHODS

On the briquettes dried samples do Proximate analysis, covering the water content (ASTM D 3173-03); volatile matter (ASTM D 3175-0); ash content (ASTM D 3174-02) and the heat content (ASTM D 2015-96). Afterwards, test the best total value category. The best treatment is selected from the category of the best total value, then used for testing flame, durability and stability. Variations in the composition of charcoal briquettes can be seen in Table - 1.

Tabel 1. Variasi komposisi briket arang.

No.	Kode Briket	Komposisi
1.	K1M10	4,5 g Plastik; 4,5 g Karet; 10 g Perekat; 81 g Arang tempurung kelapa lolos ayakan 10 *mesh*
2.	K1M20	4,5 g Plastik; 4,5 g Karet; 10 g Perekat; 81 g Arang tempurung kelapa lolos ayakan 20 *mesh*
3.	K1M40	4,5 g Plastik; 4,5 g Karet; 10 g Perekat; 81 g Arang tempurung kelapa lolos ayakan 40 *mesh*
4.	K2M10	2,25 g Plastik; 2,25 g Karet; 10 g Perekat; 85,5 g Arang tempurung kelapa lolos ayakan 10 *mesh*
5.	K2M20	2,25 g Plastik; 2,25 g Karet; 10 g Perekat; 85,5 g Arang tempurung kelapa lolos ayakan 20 *mesh*
6.	K2M40	2,25 g Plastik; 2,25 g Karet; 10 g Perekat; 85,5 g Arang tempurung kelapa lolos ayakan 40 *mesh*

RESULT AND DISCUSSION

Water Content

The water content of the entire sample of charcoal can be seen in Figure 1.

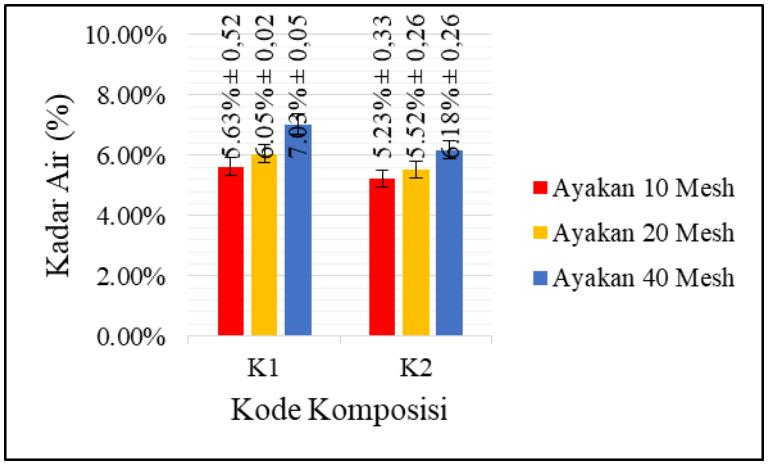

Figure 1. Levels of air samples charcoal briquette.

In Figure -1 note that the average percentage of charcoal briquettes moisture content of the first composition on sieve 10, 20, and 40 mesh was 5.63% ± 0.52; 6.05% ± 0.02; and 7.03% ± 0.05; while the second composition on a sieve of 10, 20, and 40 mesh was 5.23% ± 0.33; 5.52% ± 0.26; and 6.18% ± 0.26. The increase in the average percentage of water content of the charcoal briquettes influenced by particle size, wherein the smaller the particle size, resulting in increasingly dense bonding between the particles that occur so that the water content in the briquettes difficult exit when dried (Riseanggara, 2008).

The percentage of water content that has been obtained is then inserted into the list of categories the value of the water content in Table - 2. The percentage of moisture content of charcoal briquettes first composition passes 10 mesh sieve amounted to 5.63% ± 0.52 5.11 value included in the interval; so we get a category value of 7.5. The same way is used for the assessment of briquettes another sample.

The water content in the briquettes is expected as low as possible in order to produce a high calorific value and the briquettes are easy in the ignition or combustion initially (Ismayana and Afriyanto, 2011). The result is a sample of briquettes that pass 10 mesh sieve composition of both the briquette with the lowest water content so as to produce the calorific value and high combustion power.

Table 2. List category value of moisture.

No.	Category Value	Moisture (%)
1.	10	<3
2.	9,5	3-3,5
3.	9	3,6-4
4.	8,5	4,1-4,5
5.	8	4,6-5
6.	7,5	5,1-5,5
7.	7	5,6-6
8.	6,5	6,1-6,5
9.	6	6,6-7
10.	5,5	7,1-7,5
11.	5	7,6-8
12.	4,5	>8

Volatile Matter

Volatile content of all samples of charcoal can be seen in Figure - 2.

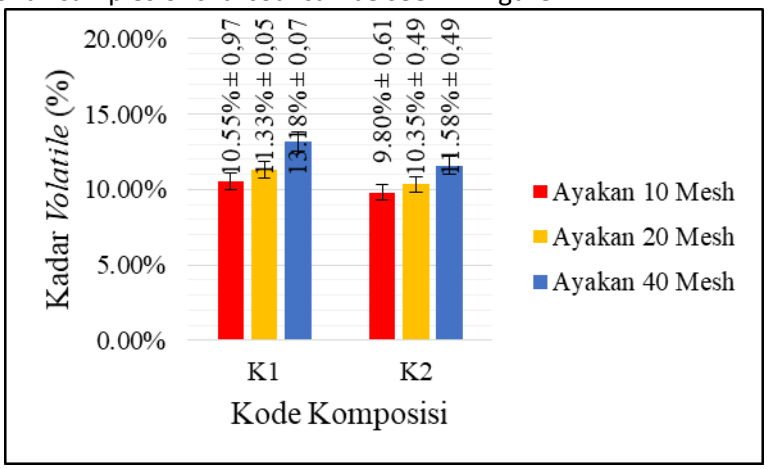

Figure 2. Levels of volatile samples charcoal briquette.

Figure 2 that the average percentage content of volatile charcoal briquettes from the first composition on sieve 10, 20, and 40 mesh was 10.55% ± 0.97; 11.33% ± 0.05; and 13.18% ± 0.07; while the second composition on a sieve of 10, 20, and 40 mesh was 9.80 ± 0.61; 10.35% ± 0.49; and 11.58% ± 0.49. Volatile levels are influenced by moisture (H2O) on charcoal briquettes because the levels of volatile help facilitate startup of a material (Solomon, 2014).

The percentage of volatile levels that have been obtained then was added to the list of categories the value of the water content in Table - 3. The percentage content of volatile charcoal first composition passes 10 mesh sieve amounted to 10.55% ± 0.97 into the interval value of 9.58; so we get a category value of 9.5. The same principle is used for the assessment of briquettes another sample.

Flying high levels of substance that would degrade the quality of the briquettes due to the number of volatile matter, the carbon content is getting smaller so that the value of the heat produced is getting low and will cause many smoke produced from burning (Ismayana and Afriyanto, 2011). The result is a sample of briquettes that pass 10 mesh sieve composition of both the briquette with the lowest levels of volatile so as to generate calorific value and high combustion power and the smoke generated when the briquettes ignited a little more because the carbon content is small.

Table 3. List category value of volatile content.

No.	Category Value	Volatille Content(%)
1.	20	<3
2.	19,25	3-3,5
3.	18,5	3,6-4
4.	17,75	4,1-4,5
5.	17	4,6-5
6.	16,25	5,1-5,5
7.	15,5	5,6-6
8.	14,75	6,1-6,5
9.	14	6,6-7
10.	13,25	7,1-7,5
11.	12,5	7,6-8
12.	11,75	8,1-8,5
13.	11	8,6-9
14.	10,25	9,1-9,5
15.	9,5	9,6-10
16.	8,75	10,1-10,5
17.	8	10,6-11
18.	7,25	11,1-11,5
19.	6,5	11,6-12
20.	5,75	12,1-12,5
21.	5	12,6-13
22.	4,25	13,1-13,5
23.	3,5	13,6-14
24.	2,75	14,1-14,5
25.	2	14,6-15
26.	1,25	>15

Ash Content

The ash content of all samples of charcoal can be seen in Figure 3.

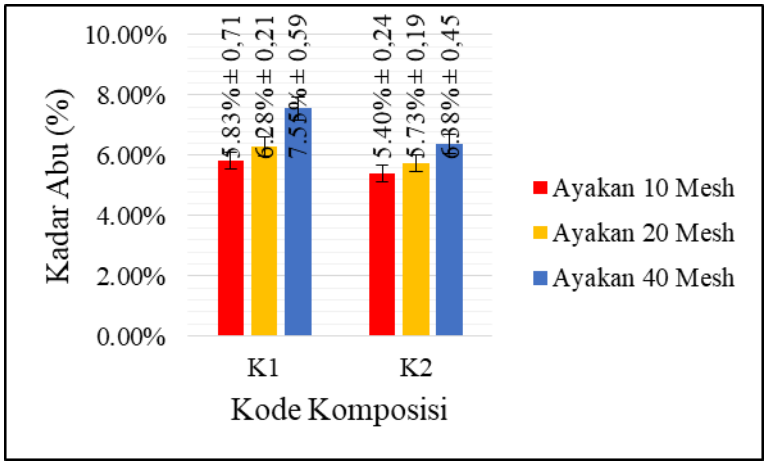

Figure 3. Levels of ash sample briquette charcoal.

In Figure 3 -note that the average percentage of ash content briquettes of the first composition on sieve 10, 20, and 40 mesh was 5.83% ± 0.71; 6.28% ± 0.21; and 7.55% ± 0.59; while the second composition on a sieve of 10, 20, and 40 mesh was 5.40% ± 0.24; 5.73% ± 0.19; and 6.38% ± 0.45. Volatile levels have an influence on the remaining ash content. The smaller the volatile content of garbage, then ash / residue generated will be less. The ash content becomes important in RDF because it affects the efficiency of combustion (Solomon, 2014).

The percentage of ash content which has been obtained then was added to the list of categories the value of the water content in Table 4. The percentage of ash content briquettes first composition passes 10 mesh sieve amounted to 5.83% ± 0.71 into the interval value of 5.12; so we get a category value of 7.5. The same principle is used for the assessment of briquettes another sample.

Ash contained in the solid fuel is a mineral that can not be burned and the remains after the combustion process of reactions that accompany it. Abu will decrease the quality of solid fuel because it can lower heating value (Jamilatun, 2011). The result is a sample of briquettes that pass 10 mesh sieve first composition; passes 10 and 20 mesh sieve composition of both the briquette with the lowest ash content based on the analysis of significance so as to produce a high calorific value due to fewer minerals are non-flammable and remains after the combustion process of reactions that accompany it.

Table 4. List category value of ash content.

No.	Category Value	Ash Content (%)
1.	10	<3
2.	9,5	3-3,5
3.	9	3,6-4
4.	8,5	4,1-4,5
5.	8	4,6-5
6.	7,5	5,1-5,5
7.	7	5,6-6
8.	6,5	6,1-6,5
9.	6	6,6-7
10.	5,5	7,1-7,5
11.	5	7,6-8
12.	4,5	>8

Calorific Value

Calorific content of the entire sample of charcoal can be seen in Figure 4.

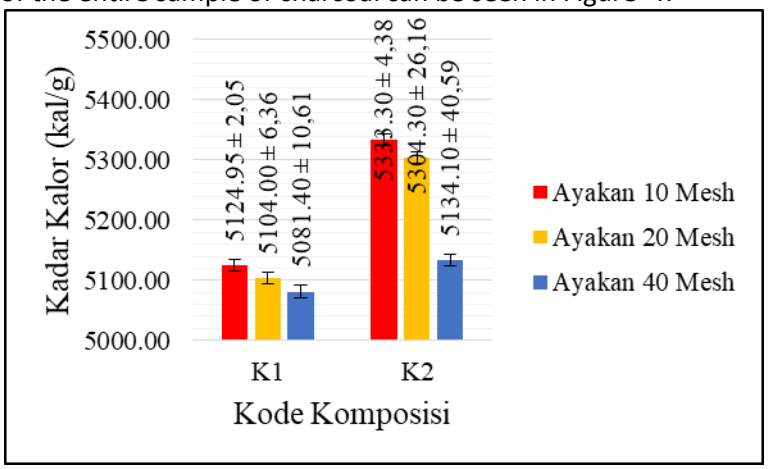

Figure 4. Content sample heat charcoal briquette

In figure 4 note that the average percentage of charcoal briquettes calorific content of the first composition on sieve 10, 20, and 40 mesh is 5124.95 cal / g ± 2.05; 5104.0 cal / g ± 6.36; and 5081.40 cal / g ± 10.61; while the second composition on a sieve of 10, 20, and 40 mesh is 5333.30 cal / g ± 4.38; 5304.3 cal / g ± 26.16; and 5134.10 cal / g ± 40.59. Volatile levels have an influence on the remaining ash content. Calorific value of RDF is the main characteristic for a good quality RDF can be seen from caloric value. Calorific value shows the energy that was conceived and produced by RDF after combustion (Solomon, 2014).

The percentage levels of heat that has been obtained is then inserted into the list of categories the value of the water content in Table - 5. Percentage of the calorific content of the first charcoal briquette composition passes 10 mesh sieve amounted to 5124.95 cal / g ± 2.05 5126.11 values included in the interval; so we get a category value of 22.5. The same principle is used for the assessment of briquettes another sample.

The lower the moisture content, volatile and ash briquettes, the greater the level of kalornya. Additionally, the power and the combustion rate is also higher so that the quality of the briquettes produced the better (Ismayana and Afriyanto, 2011). The result is a sample of briquettes that passes 10 and 20 mesh sieve composition of both the briquettes with good quality because it is based on the analysis of the significance of the sample briquettes have high combustion heat value as a fuel.

Table 5. List categories calorific value of content

No.	Category Value	Calorific Value of Content (kal/g)
1.	60	>5500 kal/g
2.	57,5	5500-5476
3.	55	5475-5451
4.	52,5	5450-5426
5.	50	5425-5401
6.	47,5	5400-5376
7.	45	5375-5351
8.	42,5	5350-5326
9.	40	5325-5301
10.	37,5	5300-5276
11.	35	5275-5251
12.	32,5	5250-5226
13.	30	5225-5201
14.	27,5	5200-5176
15.	25	5175-5151
16.	22,5	5150-5126
17.	20	5125-5101
18.	17,5	5100-5076
19.	15	5075-5051
20.	12,5	5050-5026
21.	10	5025-5001
22.	5	<5000

Total Value of Charcoal Briquettes Category

Category total value of all samples of charcoal can be seen in Table -6.

Table 6. Category value total sample charcoal briquette.

No.	Charcoal Briquette	Parameter				Total (100)
		Heat (60)	Ash (20)	Volatile (10)	Water (10)	
1.	K1M10	22,5	7,5	9,5	7,5	47
2.	K1M20	20	6,5	7,25	7	40,75
3.	K1M40	17,5	6	4,25	6	33,75
4.	K2M10	42,5	7,5	10,25	8	68,25
5.	K2M20	42,5	7,5	9,5	7,5	67,00
6.	K2M40	25	7	7,25	7	46,25

The highest total value is the composition of samples of briquettes with both the particle size of 10 mesh sieve passes by 68.25 points. The result is a sample of briquettes that pass 10 mesh sieve composition of both the sample briquette with the best quality based on the proximate analysis because it contains high levels of heat and has a volatile content; ash; as well as the lowest water from another sample of charcoal briquettes. Additionally, the power and the combustion rate is also higher.

Flame charcoal briquettes

Value flame charcoal samples can be seen in Table 7.

Tabel 7. Flame of fire value from charcoal briquette.

No.	Second compotition of charcoal briquettes with 10 mesh sieve	Length of flame until afire (minutes)	Lange of flame until become ash (minutes)	Length of ash lost (minutes)	Ash Colour
1.	Replication 1 (x1) (minute)	0,34	110,15	15,00	White
2.	Replication 2 (x2) (minute)	0,37	112,18	16,00	
3.	Average (X) (minute)	0,35 ± 0,03	111,17 ± 1,44	15,50 ± 0,71	

The rate of combustion and ignition of charcoal briquettes longer influenced by the particle size and strong press when printing. In addition, the incidence of white smoke on a charcoal briquette is influenced by the composition of coconut shell charcoal which dominated from the waste plastic and rubber.

Resilience Charcoal Briquettes

Table 8. Value Resistance samples charcoal briquette.

No.	Second compotition of charcoal briquettes with 10 mesh sieve	Lost of material (%)
1.	Replication 1 (x1) (%)	8,54
2.	Replication 2 (x2) (%)	7,87
3.	Average (X) (%)	8,21 ± 0,47

Large or small charcoal briquette material loss is affected by particle size. The smaller the particle size of the material will produce a resistance to impact which is getting stronger.

High Stability

The value of the high stability of samples of charcoal can be seen in Table 9.

Tabel 9. Value Stability samples height charcoal briquettes.

No.	Second compotition of charcoal briquettes with 10 mesh sieve	Height Changing (%)						
		Day 0 to 1	Day 1 to 2	Day 2 to 3	Day 3 to 4	Day 4 to 5	Day 5 to 6	Day 6 to 7
1.	Replication 1 (x1) (%)	1,43	0,00	0,00	0,00	0,00	0,00	0,00
2.	Replication 2 (x2) (%)	2,86	0,00	0,00	0,00	0,00	0,00	0,00
3.	Average (X) (%)	2,15 ± 1,01	0,00	0,00	0,00	0,00	0,00	0,00

Stability Diameter

Value stability charcoal briquette diameter samples can be seen in Table 10.

Table 10. Value stability samples diameter charcoal briquettes.

No.	Second compotition of charcoal briquettes with 10 mesh sieve	Diameter Changing (%)						
		Day 0 to 1	Day 1 to 2	Day 2 to 3	Day 3 to 4	Day 4 to 5	Day 5 to 6	Day 6 to 7
1.	Replication 1 (x1) (%)	2,00	0,00	0,00	0,00	0,00	0,00	0,00
2.	Replication 2 (x2) (%)	4,00	0,00	0,00	0,00	0,00	0,00	0,00
3.	Average (X) (%)	2,15 ± 1,01	0,00	0,00	0,00	0,00	0,00	0,00

Comparison with SNI 01-6235-2000

Comparison of quality of charcoal briquettes with SNI 01-6235-2000 best categories can be seen in Table 11.

Table 11. Comparison with SNI 01-6235-2000 charcoal briquette.

No.	Parameter	Best Category of Chorcoal Briquette	SNI 01-6235-2000	Information
1.	Water content (%)	5,23	Maximum 8%	Sesuai
2.	Volatile content (%)	9,80	Maximum 15%	Sesuai
3.	Ash content (%)	5,40	Maximum 8%	Sesuai
4.	Calor (Kal/g)	5333,30	Minimum 5000 Kal/g	Sesuai

Samples briquettes which passes 10 mesh sieve worthy of this second composition to be produced and marketed for the entire results of the analysis in accordance with quality requirements set by SNI 01-6235-2000 of charcoal briquettes.

CONCLUSION

The conclusion in this study is the result of the quality test of charcoal briquettes as a product of RDF, the water content of 5.267% -7.067%; volatile mater content of 9.367% -13.233%; ash content of 5.333% - 7.967%; and the calorific content of 5058.9 cal / g-5336.4 cal / g. The total value of the best of charcoal briquettes as RDF product amounted 67.00. The best treatment RDF charcoal briquettes as a product based on the total sample briquette is best that passes 10 mesh sieve second composition, which consists of 90 g made up 2.5% of plastic waste; 2.5% of the waste rubber; and 95% coconut shell charcoal. RDF charcoal briquettes as a product of the best treatments have quality test results of flame reached 111.17 minutes; resistance values of 8.21%; and stability of 2.15% and 3%. Charcoal briquettes from the category of the best total value as RDF product in accordance with the Indonesian National Standard (SNI) 01-6235-2000 based proximate analysis.%.

Briquette briquette K3 is the best because it has a total value of 87 and have parameters that meet the highest quality requirements SNI 01-6235-2000 about briquettes.

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