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Preface

We are very pleased to present proceeding of International Seminar on Tropical Bio-resources for Sustainable Bio-Industry 2013 (ISTB 2013). This seminar covered various bio-based research and applied research to support health, food, animal feed, fiber, fertilizer and fuel industries.

More than 300 participants have joined this seminar ranged of participants representing universities, research institutions, organizations, companies and government agencies from Indonesia and other countries such as the United States, Australia, Japan, Germany, Thailand, and Malaysia. Twenty-seven prominent scientists and experts in bio-based research as well as bio-based industry addressed the seminar. Additionally we have also received more than 80 full paper submissions for this seminar. Selected papers have published at Journal of Mathematical and Fundamental Sciences (ITB Journal) and Hayati Journal of Biosciences and this proceeding. It took more almost 3 years since seminar in order to make sure all published material comply with publication standard and author request.

We would like to take this opportunity to express our sincere gratitude to all honorable keynote speakers : Prof. Ferid Murad, Prof. James Dale, Prof. Eiichiro Fukusaki, Prof. Oliver Kayser, Prof. Takeshi Ohama and Prof. Shuichi Kawai. We would also like to thank all honorable invited speakers and participants who will share their experiences, ISTB Steering, Scientific Committees and Reviewers for their continuous support. Special thanks for all seminar sponsors and contributors: LPPM ITB, Rumah Sakit Hasan Sadikin (RSHS), PT. Indofood Sukses Makmur, PT. Astra Agro Lestari, PT. New Module International, Medquest, PT. Buchi Indonesia, Biofarma, Mayasari Bakery, PT. Sewu Segar Nusantara, BPPT, NARC, and SITH Research Groups and staff members. This seminar could not have been made possible without your support and great efforts.

Bandung, 1st April 2016

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Potential Use of Purple Bacteria as Carotenoid Source in Ornamental Fish Feed

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ABSTRACT - Carotenoid in purple bacteria is potential to be used as colorant agent for ornamental fish feed. The purpose of this research was to evaluate the potency of five purple bacteria isolates, namely IR5, IR,9, JPR1, Naga1n and Lp Pasir, maintained by The Laboratory of Microbiology, Research Center for Limnology LIPI. The study was focused on characterization of isolates, analyzing the spectral absorption and carotenoid content. The result showed that, all isolates were rod-shape and Gram's negative bacteria with 72 hour of logarithmic phase. All isolates had bacteriochlorophyll-a as photosynthetic apparatus. The highest content of total carotenoid was found in isolate IR5, followed by IR9, Lp Pasir, Naga1, and JPR1, respectively. Total carotenoid in all isolates was higher than krill, shrimp, krill oil, yeast, and shrimp oil which are commonly used as colorant agent for ornamental fish. Type of caretoid mostly found in mostly found in IR5, IR9, JPR1, and Naga1 was Carotenoid rhodopinal, while Lp Pasir was dominated by carotenoid spheroidene.

Keywords: purple bacteria; photosynthetic apparatus; carotenoid; colorant agent; ornamental fish.

Introduction

Indonesia is the fifth largest country exporting ornamental fish after Czech Republic, Thailand, Japan, Singapore with the total value approximately ranging between USD 60 million and USD 65 million. In 2012, the Ministry of Marine Affairs and Fisheries noted that the gross production of ornamental fish in 2012 was 978 million individuals, which was 15.16% higher than the production target of 850 million individuals. The future production opens wide opportunities for greater expansion since Indonesia is the shelter more than 450 species of total 1,100 species of ornamental fish in the world. However, in order to improve the value of exporting ornamental fish, technologies related to production need to be developed. Singapore, for instance, has applied better technology in supplying finest ornamental fish which likely become the most favored by consumer even though Singapore has limited numbers of species and geographical space as compared to Indonesia (Antara News; Bisnis Keuangan, 2013).

The development of technology for ornamental fish in Indonesia can be divided into two major strategies: (1) improvement of the grade and quality of ornamental fish to meet the standards of international market and (2) improvement from inferior to highly valued and superior ornamental fish. The enhancement of quality of ornamental fish can be attained by applying the high quality feed to the hosts/fish. One of the significant components for highly qualified feed is carotenoids that emphasize and strengthen the colorants of ornamental fish (Lesmana and Daelami, 2010).

In the present time, carotenoids in fish feed are derived from vegetable matters e.g the flower of marigold (*Tagetes erecta* L.) (Sukarman and Chumaidi, 2010), shrimp carapace flour, and semisynthetic agents. The latest research showed particular evolvement in the production of microbe-based-carotenoid supplement from *Monascus* sp., algae, *Blakeslea trispora* (Dufosse, 2006), and *Paracoccus* sp. [Harker *et al.*, 1998; European Food Safety Authority, 2007).

Purple bacteria from the group of sulphur photosynthetic have been very useful as bioremediation agents in aquaculture (Widiyanto, 1996; Anonim, 2003). During bioremediation process, these bacteria absorb toxic H_2S gas and subsequently oxidize it during anoxygenic photosynthesis to form sulfuric substance and/or sulphate that are non toxical for aquatic life.

These bacteria have vividly strong pigments trapped in its cell membranes. This pigment consists of carotenoids and bacteriochlorophyll and acts as a system to capture the lights during the process of anoxygenic photosynthesis (Pfennig and Truper, 1991).

The Laboratory of Microbiology, Research Center for Limnology LIPI, Cibinong has collected a number of isolates from the group of purple bacteria. These bacteria were previously used as the bioremediation agents in shrimp's pond and exhibited qualified potential as the carotenoid source for feed. This study aimed to evaluate the isolates of purple bacteria which potentially perform the finest carotenoids source in ornamental fish feed.

Methodologies

Sample preparation

Purple bacteria used in this study were the collection of Microbiology Laboratory, Research Center for Limnology LIPI, Cibinong. The samples were aseptically taken from water and sediment samples of shrimp's ponds and coastal areas in Karawang and Lampung. The five isolates are coded to IR5, IR9, JPR1, Lp. Pasir, and Naga1.

Prior to the experiment, all the isolates were rejuvenated and multiplied in liquid and solid media of Sea Water Complete (SWC). The composition of the media (per L) is bacto peptone 5 g (Difco), yeast extract 1 g (Difco), gliserol 3 mL, sea water 750 mL, and distilled water 250 mL. The solid media contain similar composition to that of liquid with the addition of 15 g of bacto agar (Difco) to the 1L medium. Isolates were incubated for 3 days at ambient temperature. The supply of light was provided

by bulb powered with 40 W. Approximate distance from the light source to the culture incubated was 40 centimeters (Figure 1).

Figure 1 Incubation condition

Isolate Characterization

Characterization of bacteria includes the observation of cell shape, Gram's identification, and growth pattern. Cell shape was examined by Zeiss Axiolab microscope using 1600x magnification. Safranin or crystal violet was applied prior to microscopic observation. Gram's identification referred to Ryu's method (Powers, 1995). This method is based on the reaction of the membrane cell towards KOH 3% solution. The changes in Optical Density (OD) of cultivated bacterial stock in SWC liquid medium represent the growth pattern. The observations were carried out every 24 hours using spectrophotometer at 640 nm (Widiyanto, 1996).

Spectral Absorption

The monitoring of spectral pattern referred to the method proposed by Imhoft and Caumette (2004). The three day incubated cell culture in liquid SWC was centrifuged at 8000 rpm for 10 minutes at 4^0C. The supernatant was discharged and the pellet was washed three times with NaCl 0,85%, re-suspensed in 60% sucrose and subsequently homogenized by vortex-mixer. The spectral absorption was measured by spectrophotometer UV-Vis Genesys 10S at 300 nm to 1000 nm wavelength.

Total Carotenoid Content

Total carotenoid was analyzed using modification method of Apriantono *et al.* (1989). Carotenoid in bacterial cell was extracted four times using two different concentrations of acetone-hexane solutions. In the first extraction, solution of 40 mL acetone : 60 mL hexane was added subsequently to 20 mL of three-day-old cultured cells and stirred for 5 minutes at 30^0C. After the first extraction, the bacterial pellets were extracted three times using solution of acetone-hexane (1:1) in the same conditions. The carotenoid extracts from all extraction process were collected and dehydrated by the addition of Na_2SO_4. After filtering step, carotenoid extract was analysed by spectrophotometer at 436 nm. Beta-carotene was used as the standard solution.

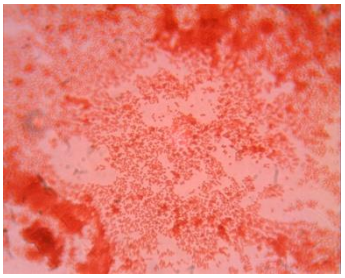
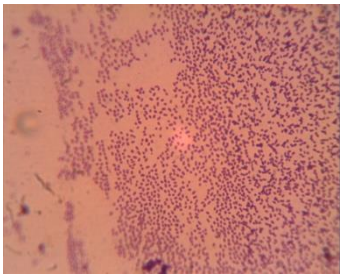
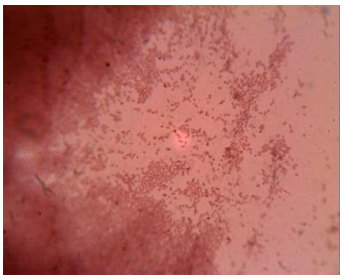
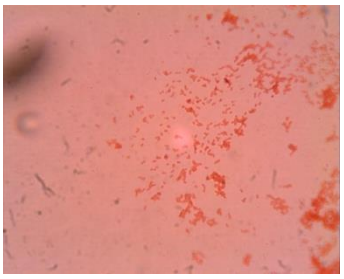
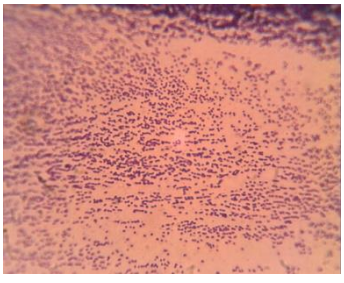
Result and Discussion

The Characteristic and Growth Pattern

Essentially, the phototrophic bacteria comprise of two different groups including purple bacteria and green sulphur bacteria. Spherical-, rod-, vibrio- or spiral-shaped are instances of the cell shapes identified from phototrophic bacteria. According to its Gram's reaction, all phototrophic bacteria are

Gram-negative (Pfennig and Truper, 1974). All purple bacterial isolates used in this study showed rod-shaped cells with Gram negative reactions. Table 1 outlines the result of Gram's staining and cell-shape observations.

Table 1 Cell shape and Gram identification of purple bacteria

Isolate	Picture	Cell Shape	Gram's Reaction
IR5		Rod	Negative
IR9		Rod	Negative
JPR1		Rod	Negative
Lp Pasir		Rod	Negative
Naga1		Rod	Negative

During the incubation period, five purple bacterial isolates began to enter stationary phase after 72 hours (Figure 2). At that time, the greatest value of OD was produced by isolates IR5, followed by IR9, Naga1, Lp Sand, and JPR1 respectively.

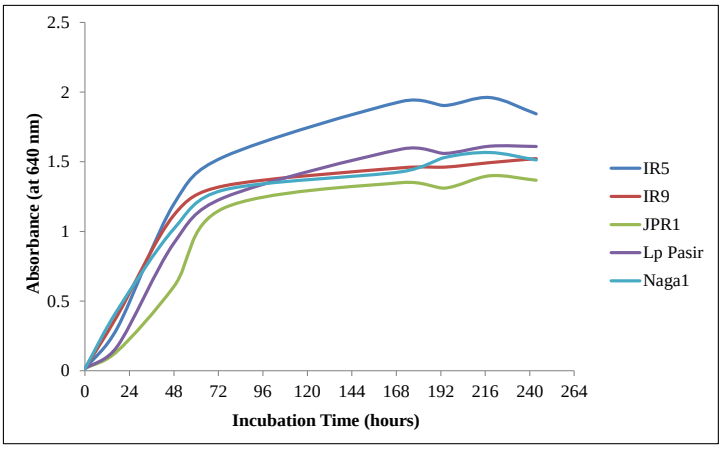


Figure 2 The growth curve of isolates IR5, IR9, JPR1, Lp Pasir, and Naga1

Spectral Pattern

As anoxygenic phototrophic bacteria, purple bacteria phototrophically grow without producing oxygen. Phototrophic bacteria are equipped by special cellular machinery, called photosynthetic apparatus, to generate anoxygenic photosynthetic process. This apparatus involves of a membrane-bound complex containing reaction center and light harvesting (antenna) pigment-protein complexes. The proteins of reaction center and antenna noncovalently bind bacteriochlorophyll, carotenoids and other cofactors in stoichiometric ratios (Imhoff, 2006). There are four major groups of bacteriochlorophyll (i.e. bacteriochlorophyll-a, -b, -c, and –d) found in phototrophic bacteria in which bacteriochlorophyll-a and –b are predominant in purple bacterial group. Absorption bands of bacteriochlorophyll-a *in vivo* are at 375, 590, 805, and 830-890 nm, whereas bacteriochlorophyll-b absorbs the wavelengths at 400, 605, 835-850, 1020-1040 nm (Pfennig and Truper, 1974). Absorption bands of IR5, IR9, JPR1, Lp Pasir, and Naga1 are illustrated in Figure 3. According to the data, all isolates performed bacteriochlorophyll-a in the photosynthetic apparatus since they had absorption bands at 375, 585, 805, and 855 nm.

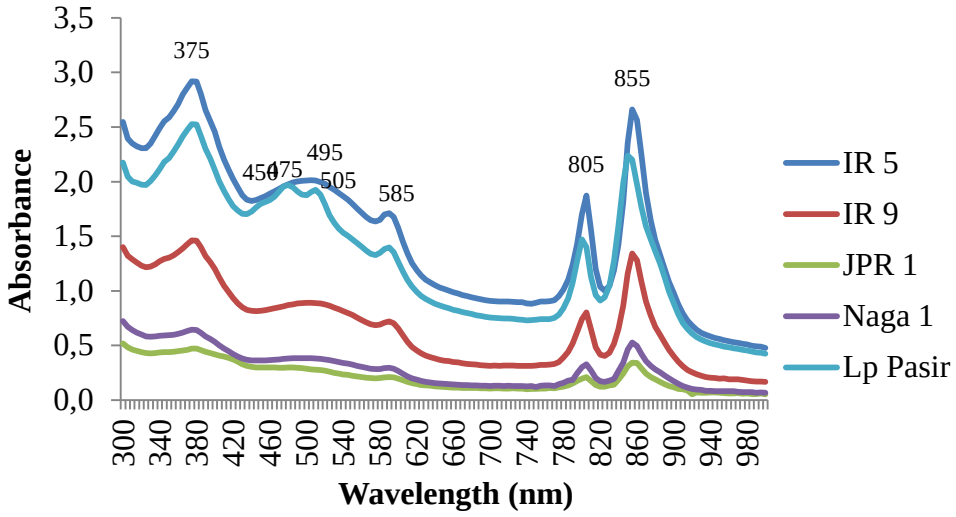


Figure 3 Absorption bands of intact cells in 60% sucrose solution

Isolate IR5, IR9, JPR1, and Naga1 had five peaks absorption at the wavelengths between 300-1000 nm, while isolate Lp Pasir had seven peaks. This difference was found at the wavelengths between 400-500 nm, in which the former group only had single peak at 495 nm, in contrast, Lp Pasir had three peaks at 450, 475 and 505 nm (Table 2).

Table 2 Peak numbers of each isolate at the wavelengths between 300-1000 nm

Isolate	Wavelength (nm)								Peaks Number
	1	2	3	4	5	6	7	8	
IR5	375	-	-	495	-	585	805	855	5
IR9	375	-	-	495	-	585	805	855	5
JPR1	375	-	-	495	-	585	805	855	5
Lp Pasir	375	450	475	-	505	585	805	855	7
Naga1	375	-	-	495	-	585	805	855	5

The difference among the isolates might be due to the difference in carotenoid content of photosynthetic apparatus. There are six classes of carotenoids in purple bacteria (lycopene and rhodophin; spirilloxanthin; spheroidene; rhodopinal; okenone; 1,2-dihydro-derivatives of lycopene and neurosporene) which have maximum absorption around 420-550 nm (Pfennig and truper, 1991). Isolate IR5, IR9, JPR1, and Naga1 had a maximum absorption at 495 nm that might be as a result of a highnumber of rhodopinal in their membranes as shown by the appearance of purple-violet color (Figure 4) (Imhoff, 2006). Isolate Lp Pasir whose culture appeared brownish red, might produce spheroidene as the major carotenoid. This was likely identified by its maximum absorption that resembled the maximum absorption of spheroidene (450, 482, and 514 nm) (Figure 4) (Pfennig and Truper, 1991).

Figure 4 Liquid culture of IR5, IR9, JPR1, Naga1, and Lp Pasir (left to right)

Carotenoid Content

Total carotenoid content of the five isolates varied from 1120 to 4950 mg/L culture. Isolate IR5 had the highest content followed by IR9, Lp Pasir, Naga 1, and JPR1 respectively (Table 3).

Table 3 Content of total carotenoid in purple bacteria

No.	Isolate	Total Carotenoid (mg/L)	Optical Density (at 640 nm)
1	IR5	4.940	1.691
2	IR9	2.560	1.634
3	Lp Pasir	1.720	1.608
4	Naga1	1.380	1.478
5	JPR1	1.120	1.477

Their concentrations were greater than those originated from the krill, shrimp, krill oil, yeast, and shrimp oil which are frequently utilized as fish feed ingredient (Lesmana and Daelami, 2009; Sukarma and Chumaidi, 2010).

Maulid (2011) and Kurnia *et.al.* (2010) observed that adding carotenoid from purple bacteria and marine bacteria Paracoccus sp. to fish feed could significantly enhance the coloration of rainbow fish and red sea Bream. Some studies had revealed the biological function of carotenoids such as, anti-inflammatory, antioxidants, anti-carcinogenic agents, species-specific colorant, prevention of chronic diseases, vision, and cellular growth and development (Lee and Schmidt-Dannert, 2002; McGraw and Ardia, 2003).

Moreover, Vrati, Kobayashi, and Kurata in Banarjee *et.al.* (2000) reported that phototropic bacteria contained 40-60% protein and essential amino acid composition comparable with egg, algae, and soybean. The study of Banarjee *et.al.* (2000) and Shapawi *et.al.* (2012) concluded that nutrition value of purple bacteria promoted the quality of feed and could improve the growth rate, survival, and feed convertion ratio in fish.

Conclusion

The five isolates of local purple bacteria maintained by Research Center for Limnology LIPI, Cibinong provide great opportunity to be used as coloring agent due to their notable they carotenoid content. Isolate IR5 is likely the most potential since it has the highest carotenoid content.

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Reference

http://www.antaranews.com/berita/370500/produksi-ikan-hias-tembus-978-juta-ekor

http://bisniskeuangan.kompas.com/read/2013/04/22/07521679/Potensi.Ekspor.Ikan.Hias.Capai.Rp.600.Miliar

Lesmana, D.S., Daelami, D. *Panduan Lengkap Ikan Hias Air Tawar*, Penebar Swadaya, 229-254, 2009.

Sukarman, Chumaidi. *Bunga tai kotok* (*Tagetas sp.*) *sebagai sumber karotenoid pada pakan ikan hias*, in Prosiding Forum Inovasi Teknologi Akuakultur, Fahmi, M.R (ed.), pp. 803 – 807, 2010.

Dufosse, L. Microbial production of food grade pigment. *Food Technology and Biotechnology* 44(3): 313-321, 2006.

Harker, M., Hirschberg, J., Oren, A. *Paracoccus marcusii* sp. Nov., an orange Gram-negative coccus. *International Journal of Systematic and Evolutionary Microbiology* 48: 543-548, 1998.

European Food Safety Authority, Scientific opinion of the Panel on Additives and Products or Substances used in animal feed on safety and afficacy Panaferd-AX (red carotenoid-rich bacterium Paracoccus carotinifaciens) as feed additives for salmon and trout. *The EFSA Journal* 546: 1-30, 2007.

Widiyanto, T. *Bakteri Fotosintetik Anoksigenik sebagai biokondisioner di tambak udang: Pengurangan produksi H_2S dan pengaruhnya pada pertumbuhan Vibrio harveyi*. [Magister thesis]. Bogor: Program Pascasarjana, Institut Pertanian Bogor, 1996.

Anonim, Laporan Teknis, Pusat Penelitian Limnologi LIPI, pp. XIV 1-40, 2003.

Pfennig, N. & Trüper, H.G. *The family Chromaticeae*, The Prokaryotes Vol. IV, Dworkin, M., Falkow, S., Rosenberg, E., Scheifer, K.H., Stackebrandt, E., (Eds.), pp. 3200-3221, 1991.

Powers, E. Efficacy of the Ryu Nonstaining KOH Technique for Rapidly Determining Gram Reactions of Food-Borne and Waterborne Bacteria and Yeasts. *Journal of American Society for Microbiology* 61: 3756-3758, 1995.

Imhoff, J.F., & Caumette, P. Recommended Standards for the Description of New Species of Anoxygenic Phototrophic Bacteria. *International Journal of Systematic and Evolutionary Microbiology* 54: 1415-1421, 2004.

Apriyantono, A., Fardiaz, D., Puspitasari, N.L., Sedarnawati, Budiyantono, S. *Petunjuk Laboratorium Analisis Pangan,* Penerbit Institut Pertanian Bogor. 1989.

Pfennig, N., Trüper, H.G. *The Phototrophic Bacteria*, Bergey's Manual of Determinative Bacteriology 8th Edition, Buchanan, R.E. and Gibbon, N.E. (eds.), The Williams & Wilkins Company, pp 24 – 51, 1974.

Imhoff, J.F. *The Phototrophic Alpha-Proteobacteria*, The Prokaryotes Volume V 3rd Edition, Dworkin, M., Falkow, S., Rosenberg, E., Scheifer, K.H., Stackebrandt, E. (eds.), pp 41-64, 2006.

Imhoff, J.F. *The Chromaticeae*, The Prokaryotes Volume VI 3rd Edition, Dworkin, M., Falkow, S., Rosenberg, E., Scheifer, K.H., Stackebrandt, E. (eds.), pp 846-873, 2006.

Maulid, M.A. *Penambahan Karotenoid Total dari Bakteri Fotosintetik Anoksigenik Pada Pakan Untuk Perbaikan PenampilanIkan Pelangi Merah (Glossolepis incisus) Jantan*. [Skripsi]. Bandung: Fakultas Perikanan Dan Ilmu Kelautan, Universitas Padjadjaran, 2011.

Kurnia, A., Satoh, S. and Hanazawa, S. Effect of *Paracoccus* sp. and Their Genetically Modified on Skin Coloration of Red Sea Bream. *HAYATI Journal of Biosciences* 17(2): 79-84, 2010.

Lee, P.C., Schmidt-Dannert, C. *Metabolic Engineering Towards Biotechnological Production Of Carotenoids In Microorganisms,* Applied Microbiology and Biotechnology 60: 1-11, 2002.

McGraw, K.J., Ardia, D.R. Carotenoids, Immunocompetence, and The Information Content of Sexual Colors: An Experimental Test. *The American Naturalist* 162(6): 704-712, 2003.

Banarjee, S., Azad, S.A., Vikineswari, S., Selvaraj, O.S. and Mukherjee, T.K. Phototrophic Bacteria As Fish Feed Supplement. *Asian-Australasian Journal of Animal Sciences* 13 (7): 991-994, 2000.

Shapawi, R., Ting, T.E., Al-Azad, S. Inclusion of Purple Non-Sulfur Bacterial Biomass in Formulated Feed to Promote Growth, Feed Conversion Ratio and Survival of Asian Seabass Lates Calcarifer Juveniles. *Journal of Fisheries and Aquatic Sciences* 7(6): 475-480, 2012.

Optimization of Lovastatin Production Through Fermentation Process of Rice Bran and Tofu Waste by *Monascus purpureus*

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ABSTRACT - Rice bran and tofu waste are food production byproducts in which their annual production are quite high in Indonesia. The usage of rice bran and tofu waste is usually for raw material for animal feed. The value of those byproducts can potentially increased through fermentation process. One of the added values is producing lovastatin by fermentation process using *Monascus purpureus*. Lovastatin is a compound that can be used to inhibit cholesterol synthesis in human and animal. The aim of this study is to obtain the composition of rice bran and tofu waste that produce optimum quantity of lovastatin. Inoculum used in this fermentation is 10% (v/w) spore inoculum of 108 hours *M. purpureus* spore with the density of 10^7 spores/ml. Fermentation process is carried out for eight days at the temperature of 30^oC with ratio of rice bran and tofu waste 5:5, 6:4, 7:3 and 8:2. Variations in percentage of initial water content are 50%, 60%, 70% and 80%. The quantity of lovastatin is measured by UV-VIS spectrophotometer and High Performance Liquid Chromatography (HPLC). Analysis of lovastatin production using UV-VIS spectrophotometer is conducted at wavelength of 370 nm and lovastatin is extracted using ethanol:water (75:25). While, in analysis using HPLC method, lovastatin from sample is extracted using ethyl acetate and acetonitrile. The highest amount of lovastatin produced, 341.7301 μg/g, obtained from ratio of rice bran and tofu waste 5:5. Meanwhile, the optimum initial water content is 50% which produced lovastatin up to 805.745 μg/g. Based on this results, it can be concluded that highest production of lovastatin is obtained from fermentation process of rice bran and tofu waste ratio of 5:5 and 50% initial water content.

Key word: lovastatin, *Monascus purpureus*, rice bran, tofu waste

Introduction

Rice bran is food production byproducts in which their annual production are quite high in Indonesia. Rice bran is widely used for animal feed mixture but it still contains about 65% of total available micronutrients in rice. Nutrition found in rice bran include carbohydrates, protein, vitamin B and some mineral. Of the overall content of the nutrients found in rice bran, carbohydrate was the highest content.

Other food waste produced in high amount is tofu waste. It is known that the tofu waste produced per yer reach until 731,501.5 tonnes in Indonesia and 48.153 tonnes in West Java. Tofu waste has a qite high protein content of the amino acids lysine and methionine and high calcium (Mahfudz, 2006; Yuslinawati, 2006).

The value of those byproducts could be added through fermentation process. Nuraini *et al.* (2012) showed that fermented rice bran and tofu waste by *Monascus purpureus* can be used to produce lovastatin and used as a feed mixture that has added value because it can lower cholesterol levels in livestock that consume the fermented feed. Lovastatin can lower cholesterol level by preventing oxidation of lipids and inhibit the action of HMG Co-A reductase competitively so mevalonate are needed for cholesterol synthesis not formed (Rusli, 2011).

Rice bran is a common carbon source used for lovastatin production by fungi. In addition, rice bran are also known to contain quite high protein and essential amino acids (Saputra, 2008) and addition of organic nitrogen sources can increase the production of lovastatin (Hajjaj *et al*., 2001). One of substrates that can be used as an organic nitrogen source is tofu waste. However, in order to get nutritional balance needed for growth and metabolite production by microbes, perfect combined rice bran and tofu waste as a substrate for fermentation should be obtained first. Furthermore, correct initial moisture content of the substrate is also a very important parameter to regulate microbial growth and metabolite production mainly on solid fermentation (solid state fermentation). Generally, the optimum initial water content for lovastatin production by *M. purpureus* is about 60 to 70% (Ganrong *et al.*, 2003).

The aim of this study is to obtain the composition of rice bran and tofu waste and intial water content that can produce optimum quantity of lovastatin. Variations of ratio rice bran:tofu wastre are used in this experiment are 5:5, 6:4, 7:3 and 8:2. Variations in percentage of initial water content are used in this experiment are 50%, 60%, 70% and 80%.

Materials and Methods

Determination of the best spore's age as source of inoculum *Monascus purpureus*

Inoculum of *M. purpureus* in PDA medium initially dissolved using 0.85% NaCl and 0.1% Tween 80. Spore suspension with the density of 10^6 spores/mL were inoculated in 13 test tubes medium containing PDA (Poteto Dextrose Agar) medium. Each tube was sampled every 12 hours for 144 hours. Each sampling conducted by making spore suspensions using 0.85% NaCl and 0.1% Tween 80. The concentration of spore suspension are counted using a haemocytometer and about 1 mL of spore suspension is then taken for Total Plate Count (TPC). Number of viable colonies are calculated after 48 hours incubation. The entire experiment was carried out aseptically and duplicate. Age of spores with the highest viability was then used as a source of inoculum in subsequent experiments. Viability of spores was calculated by the formula (Herlinda *et. al.*, 2006):

$$\% \text{ Spore Viability} = \frac{amount\ of\ viable\ \ colony\ quantified\ by\ TPC}{amount\ of\ spore\ quantifed\ by\ haemocytometer} \times 100\%$$

Determination of the best ratio of rice bran : tofu waste in fermentation by *Monascus purpureus*

Rice bran and tofu waste with ratio 5:5, 6:4, 7:3, dan 8:2 was added with aquades (water content 70%) and sterilized for 30 menit with boiling water. After sterilization, 10% v/w *Monascus purpureus* spore suspension with the density of $4x10^7$ spora/mL from the best spore's age that have the highest viability was inoculated in substrate and incubated for 8 days at 30^0C (Nuraini *et. al.*, 2012). Substrate acidity (pH), enzymatic activity (by fluorescein diacetate hydrolytic method), and the amount of lovastatin produced (by spectrophotometric and high performance liquid chromatography method) was measured every 24 hours. Proximate analysis (crude nutrient analysis) was carried out at beginning and end of fermentation process.

Determination of the best initial water content in fermentation by *Monascus purpureus*

Rice bran and tofu waste with ratio 5:5 was added with aquades, to create variation of initial water content, and sterilized for 30 menit with boiling water. Variations in percentage of initial water content are 50%, 60%, 70% and 80%. After sterilization, 10% v/w *Monascus purpureus* spore suspension with the density of $4x10^7$ spora/mL from the best spore's age that have the highest viability was inoculated into substrate and incubated for 8 days at 30^oC (Nuraini *et. al.*, 2012). Initial water content was calculated by the formula (Ganrong *et. al.*, 2003):

$$\frac{wet\ weight - dry\ weight}{wet\ weight} x\ 100\% = \%\ water\ content$$

Substrate acidity (pH), enzymatic activity (by fluorescein diacetate hydrolytic method), and the amount of lovastatin produced (by spectrophotometric and high performance liquid chromatography method) was measured every 24 hours. Proximate analysis (crude nutrient analysis) was carried out at beginning and end of fermentation process.

Result and Discussion

Determination of the best spore's age as source of inoculum *Monascus purpureus*.

Viability spore curve of *M. purpureus* (Figure 1) shows the best spore's age was 108 hours with highest viability 0.28%. Low spore viability of *M. purpureus* could be caused by several things, such as (i) the medium used is not suitable. PDA is a nutrient-rich medium, but too many nutrients can inhibit spore formation in fungi (Herlinda et. al., 2006). Too many nutrients, that exceed the needs of fungi, could cause a buildup of metabolites that can inhibit the metabolism of fungi reproduction (Alberts *et al*., 1994 in Herlinda *et al*., 2006). Then (2) frequent subculture could decrease spore viability due to reducing density of fungal spores (Herlinda *et. al.*, 2006).

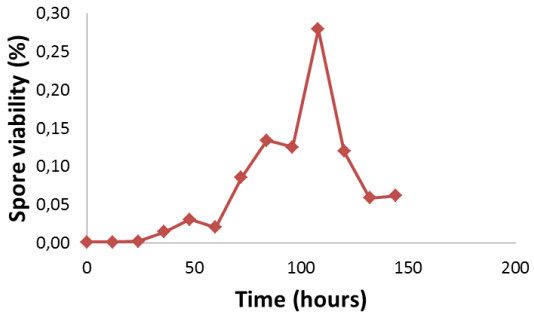

Figure 1 Spore viability of *Monascus purpureus*
(initial pH 5,6; incubation temperature 30^0C; spore inoculum 10^6; without agitation)

Determination of the best ratio of rice bran : tofu waste in fermentation by *Monascus purpureus*

Analysis of enzymatic activity during fermentation (Figure 2) showed that highest enzymatic activity found in substrate of ratio of rice bran:tofu waste 5:5 with the highest activity recorded on day 8 was 108,0956 µM/g/h and rate of activity of 40,9206 µM/g/day. The highest enzymatic activity is in the ratio 5:5 because vitamins, minerals, and trace elements balance contained in the substrate needed by fungi for fermentation process is more suitable than the other variations.

C/N ratio at each variation was calculated based on the result of proximate analysis of rice bran and tofu waste and indicate that all variations have a value of C/N ratio of about 3 (Table 1). Value of C/N ratio for support the growth of fungi is approximately 7 to 9. In this study, low C/N ratio of substrate created stress to *M. purpureus* in which could initiated secondary metabolites production from the initial fermentation. *Monascus purpureus* can grow in the environment with low C/N ratio because in addition to providing a source of carbon and nitrogen, rice bran and tofu also provides many other ingredients that can support the growth of fungi such as essential amino acids, some vitamins, and other micronutrient content.

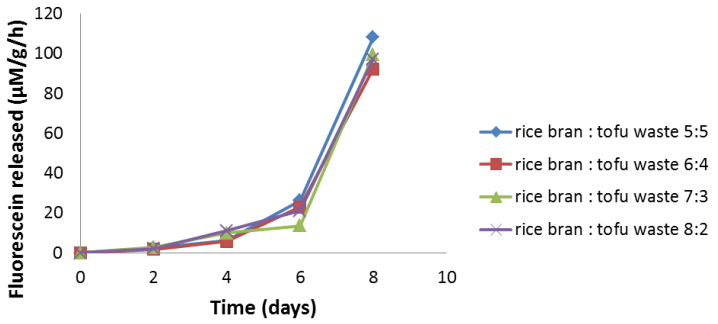

Figure 2 Enzymatic activity of *Monascus purpureus* in fermentation with different ratio of rice bran:tofu waste

Table 1 C/N Ratio in fermentation with different ratio of rice bran:tofu waste

Variation	Organic Carbon (%)	Organic Nitrogen(%)	C/N Ratio
5:5	0,218	0,072	3,028
6:4	0,231	0,077	3,000
7:3	0,244	0,082	2,976
8:2	0,256	0,087	2,942

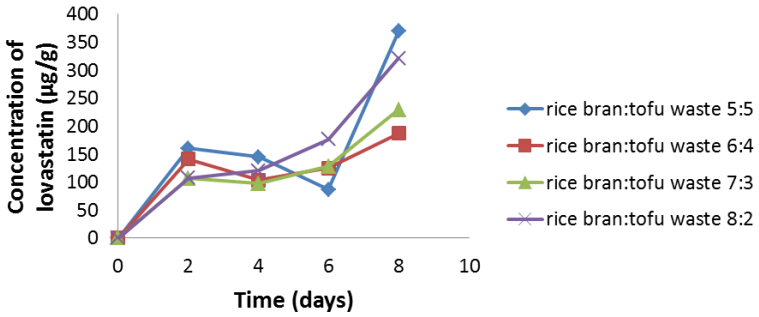

Figure 3 Lovastatin production in fermentation with different ratio of rice bran:tofu waste

Enzymatic activity correlated with lovastatin production by *M. purpureus*. The analysis using spectrophotometric method (Figure 3) and HPLC (High Performance Liquid Chromatography) method (Table 2) showed that highest production of lovastatin obtained on the ratio of rice bran:tofu waste 5:5. Concentrations of lovastatin obtained on spectrophotometric method is 369,3389 µg/g with

the highest production rate of 141,3983 µg/g/day, while the concentration of lovastatin were obtained on HPLC method is 341.7301 µg/g.

High content of lovastatin occurs on ratio rice bran:tofu waste 5:5 because the C/N ratio is highest in the variation 5:5. Besides, the balance of vitamins, minerals, and trace elements is expected has been reached in the variation 5:5. Carbon source plays an important role in the complex regulation of gene expression and enzyme activity for polyketide synthesis (Hajjaj *et. al*., 2001). In addition, organic nitrogen has important role for the production of lovastatin. Hajjaj *et. al.* (2001) and Ahmed *et. al.* (2012) found that the addition of organic nitrogen sources on the substrate can increase the production of lovastatin by fungi.

During fermentation, there was similar pattern of substrate acidity all substrate variations (Figure 4). pH value of substrate on early days of fermentation until day 2 was decreasing then the pH value continues to increase on day 2 until day 8. Decrease of pH value at the beginning of fermentation occurs due to the consumption of carbon sources such as sugars produce acidic metabolites. After carbon source exhaust, fungi began to utilize nitrogen sources on the environment and produce alkaline compounds that increased of pH value (Hajjaj *et. al.*, 2001).

Table 2 Amount of Lovastatin production by fermentation with different ratio of rice bran:tofu waste

Variation	Area	Concentration of Lovastatin (µg/g)
5:5	205360	341,7301
6:4	147885	206,9122
7:3	279866*	123,2601
8:2	302842*	142,5979

NB: Sign (*) indicates the sample was injected into the HPLC machine as much as 20µL while the other sempel as 5µL

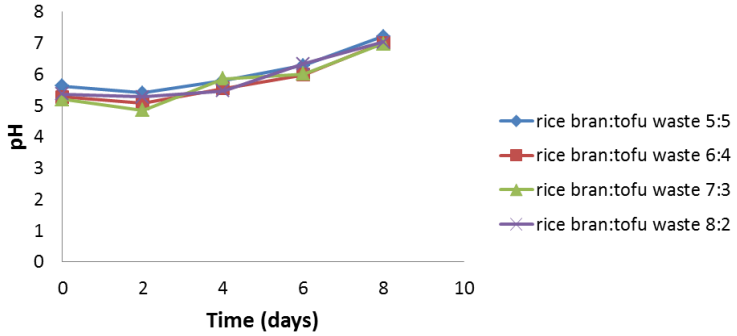

Figure 4 pH alteration in fermentation with different ratio of rice bran:tofu waste

Determination of the best initial water content in fermentation by *Monascus purpureus*

Initial water content is also a key parameter to affect the growth of microorganisms and metabolite production of *M. purpureus* especially for solid-state fermentation (Ganrong *et. al*., 2003). Analysis of enzymatic activity during fermentation with variation in initial water content (Figure 5) showed that the highest enzymatic activity seen in the initial water content of 50% with the highest activity is on day 8 of 40,8752 µM/g/h and rate of activity of 8,1248 µM/g/day.

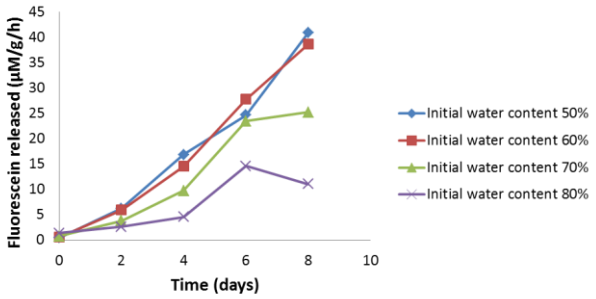

Figure 5 Enzymatic activity of *Monascus purpureus* during fermentation with different intial water content

Table 3 Amount of Lovastatin production by fermentation with different intial water content

Variation	Area	Concentration of Lovastatin (µg/g)
50%	403177	805,745
60%	365493	717,3505
70%	350509*	341,1014
80%	330555*	317,6986

NB: Sign (*) indicates the sample was injected into the HPLC machine as much as 20µL while the other sempel as 5µL

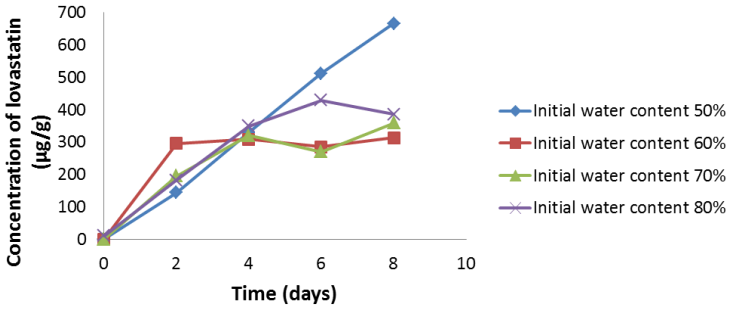

Figure 6 Lovastatin production in fermentation with different intial water content

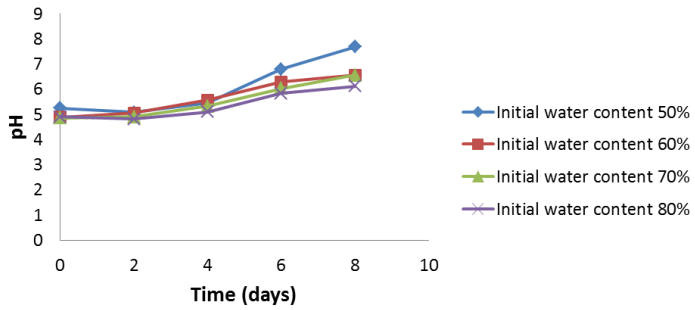

Figure 7 pH alteration in fermentation with different intial water content

Amount of lovastatin production analyzed by spectrophotometric method (Figure 6) and HPLC (*High Performance Liquid Chromatography*) method (Table 3) shows that the highest lovastatin production obtained at initial moisture content of 50%. Concentrations of lovastatin obtained on spectrophotometric method is 666,1356 µg/g with the highest production rate is 77,3729 µg/g /day, while the concentration of lovastatin obtained on HPLC method is 805.745 µg/g.

The highest lovastatin production obtained at initial moisture content of 50% due preference of *M. purpureus* to grow on "not too moist" environment. Higher moisture level decreased porosity and reduced oxygen transfer (Prabhakar *et al.*, 2012), while oxygen molecule are essential for the biosynthesis of polyketide on solid fermentation (Dhale, 2007). Lovastatin is fungal metabolite produced by the polyketide pathway (Praveen *et. al.*, 2012).

Changed in substrate pH showed a similar pattern with previous measurement on research of variation ratio of rice bran: tofu waste (Figure 7). pH value decrease at the beginning of fermentation process to day 2. At the beginning of fermentation, fungi consume carbon sources such as sugars and the main fermentation products were ethanol, organic acid, and CO_2, while acetate was produced at a very low level. Then the pH began to increase on day 2 to day 8 of fermentation. Increased of pH value caused by fungi begin to utilize nitrogen sources on the environment and produce substances that are alkaline. When the fungi begin to utilize this nitrogen, acetate was produced at a higher level. Higher acetate production resulted higher production of lovastatin since lovastatin molecules synthesized from acetate (Hajjaj *et. al.*, 2001; Seenivasan *et. al.*, 2008).

Proximate analysis result

Table 4 Proximate analysis result in fermentation with ratio of rice bran:tofu waste 5:5

Ingredient	Before Fermentation (%)	After Fermentation (%)
Protein	9,9449	10,0765
Starch	45,2238	47,1515
Crude fibre	20,2358	20,1961
Lipid	6,8452	6,3204
Ash	9,6248	9,5801,

Table 5 Proximate analysis result in fermentation with initial water content 50%

Ingredient	Before Fermentation (%)	After Fermentation (%)
Protein	8,5432	9,5958
Starch	48,5222	50,1141
Crude fibre	21,9124	20,3125
Lipid	6,2130	5,0639
Ash	8,7391	8,6801

Table 6 Proximate Analysis Result for Rice Bran and Tofu waste

Analysis	Content (%)	
	Rice bran	Tofu waste
Protein	9,6436	4,7649
Starch	28,1968	15,3974
Crude fibre	31,4335	26,9634
Lipid	4,1504	0,3204
Ash	15,8463	0,7913

Proximate analysis performed on the fermented product with the highest lovastatin production. Proximate analysis results of the first fermentation with ratio rice bran:tofu waste 5:5 (Table 4) and the second fermentation with initial moisture content of 50% (Table 5) showed similar results. Fermentation increased protein and starch content and lower fiber, fat, and ash of the substrate. Increased the value of protein occurred because growth of fungal biomass that containing protein (Takahashi *et. al.*, 2010). Increased starch content is occured due to the addition of fiber degradation. Decreased in fat content after fermentation is due to enzymes produced by fungi that can hydrolyze fat. In addition, decreased in crude fiber content in the fermentation is due to the fiber degrading enzymes produced by fungi (Mahfudz, 2006). Fiber found in rice bran is generally in the form of cellulose and hemicellulose are then degraded by cellulase enzymes to glucose.

Proximate analysis result indicate that nutrition contained in the fermented product still can be used as raw material for feed because nutritional content of the fermentation tends to be better than the nutrition found in rice bran and tofu waste (Table 6). Crude fiber content in the fermentation was lower than in rice bran and tofu waste. The lower crude fiber content, the better when it is used as feed mixtures because crude fiber has a low level of digestibility (Mahfudz, 2006). In addition protein

and starch in fermented product is higher than in rice bran and tofu waste. Protein and starch is an important component in the feed material as the main energy source for livestock. Ash content of the fermented lower than in rice bran. The ash content represent an inorganic content thus a lower ash content will be better when used as feed mixture.

Conclusion

Through these experiments it can be concluded that optimum ratio of rice bran : tofu waste for lovastatin production by *M. purpureus* is 5:5 and initial water content of 50% that can produce lovastatin up to 805.745 μg/g. Proximate analysis results showed that fermented rice bran and fermented tofu by *M. purpureus* has the potential to be used as raw material for feed.

References

Ahmed, I.E., Roquia, A.H. Elevated yield of Lovastatin by *Monascus purpureus* from Date Waste extract and encapsulation in nanoparticles. *International Journal of Pharmaceutical Science and Health Care* 6(2): 62 – 83, 2012.

Dhale, M.A. 2007. *Physiology of M. purpureus in Relation to Metabolite Production and Application as Functional Food*. [Thesis]. India: Departement of Food Microbiology, Central Food Technological Research Institute, 2007.

Ganrong, X., Yue, C., Yun, C., Xiaorong, L., Xing, L. *Production of Monacolin K in Solid-state Fermentation of Monascus sp. 9901 that does not produce citrinin*. Jiangsu: Key laboratory of Industrial Biotechnology of Ministry of Education, School of Biotechnology in Southern Yangtze University. p. 1 – 7, 2003.

Hajjaj, H., Niederberger, P., Duboc, P. Lovastatin Biosynthesis by *Aspergillus terreus* in a Chemically Defined Medium. *Applied and Environmental Microbiology* 67(6): 2596 – 2602, 2001.

Herlinda, S., Utama, M.D., Pujiastuti, Y., Suwandi. Kerapatan dan Viabilitas Spora *Beauveria bassiana* (BALS.) Akibat Subkultur dan Pengayaan Media, serta Virulensinya Terhadap Larva *Plutella xylostella* (LINN.). *Journal Hama dan Penyakit Tumbuhan Tropika* 6(2): 70 – 78, 2006.

Mahfudz, L.D. Efektifitas Oncom Ampas Tahu sebagai Bahan Pakan Ayam Pedaging (The Effective Used of Oncom Tofu By-product as a Broiler Chicken Feedstuff). *Animal Production* 8(2): 108 – 114, 2006.

Nuraini, Latif, S.A., Djulardi, A. *Evaluation of Fermented Rice Bran-Tofu Waste by* Monascus purpureus *in the Diet on Performance and Quality of Meat Broiler*. [Proceeding of the 2nd International Seminar on Animal Industry]. Padang: Faculty of Animal Science, University of Andalas. p. 225 – 230, 2012.

Prabhakar, M., Lingappa, K., Vivek, B., Amena, S., Vishalakshi, N., Mahsesh,D. Characterization of Physical Factors for Optimum Lovastatin Production by *Aspergillus terreus* KLVB28mu21 Under Solid State Fermentation. *Journal of Recent Advances in Applied Sciences* 27: 1 – 5, 2012.

Praveen, V.K., Savitha, J. Solid State Fermentation: An Effective Method for Lovastatin Production by Fungi Over Submerged Fermentation. *E3 Journal of Biotechnology and Pharmaceutical Research* 3(2): 15 – 21, 2012.

Rusli, R.K. *Pemberian Campuran Dedak dan Ampas Tahu Fermentasi dengan* Monascus purpureus *Terhadap Performa dan Kualitas Telur Ayam*. [Thesis]. Padang: Program Studi Ilmu Peternakan Pascasarjana Universitas Andalas, 2011.

Saputra, I. *Evaluasi Mutu Gizi dan Indeks Glikemik Cookies dan Donat Tepung Terigu yang Disubstitusi Parsial dengan Tepung Bekatul*. [Skripsi]. Bogor: Departemen Ilmu dan Teknologi Pangan, Fakultas Teknologi Pertanian, Institut Pertanian Bogor, 2008.

Seenivasan, A., Subhagar, S., Aravindan, R., Viruthagiri, T. Microbial Production and Biomedical Applications of Lovastatin. *Indian Journal of Pharmaceutical Science* 70(6): 701 – 709, 2008.

Takahashi, J.A., Carvalho, S.A. Nutritional Potential of Biomass and Metabolites from Filamentous Fungi. *Technology and Education Topics in Applied Microbiology and Microbila Biotechnology* 2010: 1126 – 1135, 2010.

Yuslinawati. *Isolasi dan Karakterisasi Sifat-Sifat Fungsional Protein Ampas Tahu*. [Skripsi]. Bogor: Fakultas Teknologi Pertanian Institut Pertanian Bogor, 2006.

FEED - 03

The Effect of Different C:N Ratio to Bacterial Community in Giant Freshwater Prawn (*Macrobrachium rosenbergii* de Man) Nursery Using Zero-Water Discharge (ZWD) System

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ABSTRACT - In aquaculture, bacteria plays an important role in the process of converting organic waste. Their growth is influenced by the ratio of carbon to nitrogen (C:N) in the feed given. The purpose of this study was to determine the effect of different C:N ratio in the prawns feed on bacterial communities in ZWD system. The study was conducted using two different C:N ratio in the shrimp feed, i.e. C:N 11 and C:N 7.5. Several bacteria showed in both treatments during the culture period, *Bacillus megaterium*, *Bacillus cereus*, *Pseudomonas* sp., *Staphylococcus* sp., *Flexithrix* sp., *Bacillus* sp.1, nitrifying bacteria and five other isolates that have not been identified. Total heterotrophic bacteria (THB) in the pond C:N 11 and C:N 7.5 respectively were10^8 and10^9 CFU/mL. Total nitrifying bacteria (TNB) increased during the culture period until 10^7 CFU/mL for both treatments. THB and TNB numbers were not significantly different towards the C:N ratio, but significantly different to the period of prawn culture. The stability of bacterial communities in ponds treated with C:N 11 and C:N 7.5 were 60% and52% respectively. Based on these results, it can be concluded that C:N ratio of the prawns feed had no effect to the total bacterial population in the nursery. Furthermore, the application of ZWD and nitrifying bacteria as the addition toC:N11treatment provided more stable bacterial community compare to C:N 7.5 treatment in six weeks observation period.

Keywords: aquaculture, bacterial community, giant freshwater prawn, C:N ratio, zero-water discharge

FULL PAPER WITHDRAWED by AUTHOR

Effect of Supplementation of Sucrose and Ammonium Nitrate to Citric Acid Production by *Aspergillus niger* in Solid State Fermentation of Pineapple Peel

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ABSTRACT - Pineapple peel was used as a substrate for citric acid production by *Aspergillus niger* using solid state fermentation method. Experiments were carried out at 30^{o}C with the presence of methanol 4% (v/w) and 70% moisture level as fixed variables. Fermentation process was conducted in the absence and presence of sucrose and ammonium nitrate at different concentration. In the absence of sucrose and ammonium nitrate the optimum citric acid production (4.48 g/kg) was obtained at the 5th day of fermentation. In the first stage, medium was supplemented by sucrose at different concentration. It was found that pineapple peel with addition of 14% (w/v) sucrose produced optimum citric acid (8.512 g/kg) and the yield is 27.46% based on the amount of sugar consumed. Moreover, in the second stage, medium was supplemented by sucrose and ammonium nitrate at different concentration. The addition of 14% (w/v) sucrose and ammonium nitrat (0.1 g/L) gave the optimum citric acid (10.752 g/kg) at the 4th day of fermentation. The yield produced based on sugar consumed with the variation of 14% (w/v) sucrose and ammonium nitrate (0.1g/L) was 28,10%.

Keywords: ammonium nitrate, *Aspergillus niger*, citric acid, pineapple, sucrose

Introduction

Citric acid is an important organic acid produced through ~~a~~ fermentation process. Global citric acid production in 2007 was estimated to exceed 1.6 million tonnes (Berovic and Leqisa, 2007). The high rate of citric acid production caused by the significant application of citric acid in many industries, one of them is cosmetic industry. A small amount of citric acid can be found in shampoo, soap, cleanser, hand soap and other cosmetic products. In addition, citric acid is also one of the AHA compounds which plays a role in the exfoliation of dead skin cells.

Pineapple (*Ananas comosus* L. Merr) is one of the important tropical fruit commodities based on the usefulness and economic value (Hadiati, 2009). According to Badan Pusat Statistik (BPS) in 2012, pineapple occupied the third largest production levels in Indonesia with total production of 1,749,817 tons. Besides consumed as fresh fruit, pineapple is also widely used in industry and households, such as raw material for syrup, essence of fermented drinks, crisps and jam. During production process, only small part of fruit body of pineapple was used and the rest, especially peels, are thrown away.

Pineapple peel waste attracted much attention of researchers to meet the high demand of citric acid. Several previous studies have shown that pineapple peel can be utilized as a substrate in the production of citric acid using a fermentation process.

A number of microorganisms, like bacteria (*Arthrobacter paraffinens*, *B. licheniformis*), fungi (*Aspergillus niger*, *Aspergillus aculeatus*, *Aspergillus awamori*) and yeasts (*Candida tropicalis*, *Candida oleophila*, *Yarrowia lipolytia*) can be used for the production of citric acid. Among the mikroorganisms, *A. niger* is the most commonly used commercially because it can produce more citric acid (Soccol *et. al.*, 2006).

It is necessary to find the appropriate ratio of C:N to increase biosynthesis of citric acid. Therefore, in this study will be examined the effect of supplementation of sugar as a carbon source and supplementation of nitrogen sources on the growth and production of citric acid using solid substrate fermentation of pineapple peel.

Material and Methods

Pre-treatment of Pineapple Peels

Peels used in the present study were obtained from pineapple bought at Setiabudi area, Bandung, Jawa Barat, Indonesia. Pineapple peels were dried in an oven at 60^{o}C for two days and then crushed using a blender.

Media Preparation

Five grams of crushed pineapple peels were put in 250 mL Erlenmeyer flask and moistened to reach the moisture level of 70%. In the absence of sucrose and ammonium nitrate, crushed peels were moistened by distilled water, whereas in the addition of sucrose and ammonium nitrate, crushed peels were moistened with the solution of sucrose and ammonium nitrate.

In the first stage of fermentation, crushed peels were supplemented with 14%, 18% and 22% (w/v) of sucrose solution. While in the second stage of fermentation, crushed peels were supplemented by sucrose solution with optimum concentration and ammonium nitrate solution with the variation of 0.1, 0.25, 0.4 g/L.

The flasks containing medium described above were sterilized at 121^{o}C for 15 minutes. After sterilization, flasks containing medium were allowed to cool at room temperature then inoculated with 1 mL of *Aspergillus niger* spores suspension ($2x10^{7}$ spores/mL). Prior to inoculation, methanol 4% (v/w) was added to the medium. Ater inoculation, flasks were incubated at 30^{o}C incubator for nine days. One flask was harvested every day and the entire content of the flask was taken as sample for the estimation of citric acid, sugar, pH and extracellular enzyme activity.

Extraction Method

Distilled water was added to fermented material (100 mL water for five grams of fermented material). The flask was agitated on a rotary shaker for 2 hours. After that, fermented material was filtered using Whatman filter paper no.1.

Determination of Citric Acid

Citric acid was determined titrimetrically using 0.1N NaOH and phenolphthalein as indicator and calculated as gr/kg of pineapple peels to the formula.

Determination of pH, Sugar Consumed and Extracellular Enzyme Activity

The pH value was measured using Analog pH meter and sugar content was measured using phenol-suphuric acid method. One gram of fermented material was used to determine extracellular enzyme activity using Fluorescein Diacetate Assay (FDA)

Result and Discussion

Solid State Fermentation of Pineapple Peels

Citric acid was produced from the first day of fermentation and continues to rise until it reached the optimum amount (4.48 g/kg) on the fifth day of fermentation and produced yield (product of the substrate) of 9.180%.

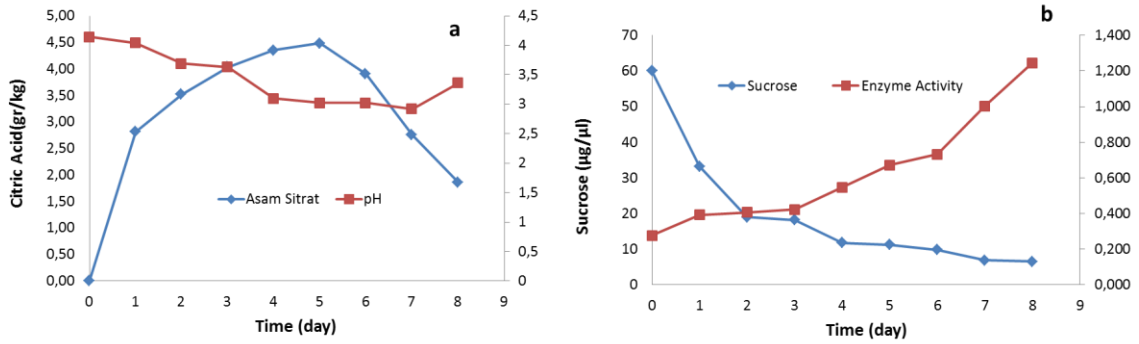

Figure 1. (a) Relation of Citric Acid Production and pH Value; (b) Relation of Sugar Consumed and Extracellular Enzyme Activity of *Aspergillus niger*
Environmental Condition : temperature of incubation 30°C, time of incubation 192h, inoculum $2x10^7$ spora/mL, initial moisture level 70%, methanol 4% (v/w), without agitation

After citric acid production capacity has reached optimum on the fifth day, the amount of citric acid decreased until the end of fermentation. Increasing citric acid production followed by decreasing pH of the medium decreased due to the higher concentration of H^+ ions. When the amount of citric acid declined, the pH tended to a constant value and began to rise until the end of fermentation process (Figure 1a). Based on Figure 1.b, the use of sugar in substrate was proportional to extracellular enzyme activity of *A.niger*. Higher extracellular enzyme activity indicated higher biomass of *A.niger*.

Solid State Fermentation of Pineapple Peels With The Variation of Sucrose

Citric acid production continued to increase and reached the optimum value in the fifth day of fermentation and eventually decreased until the end of fermentation (Figure 2.a). Citric acid produced on the addition of sucrose 14%, 18% and 22% (w/v) were 8.512, 7.104, and 7.168 g / kg of pineapple skin ,respectively, with the highest yield was 27.46% in the addition of sucrose 14% (w/v). The changes in pH value shown in Figure 2.b which also indicated the formation of citric acid. Increasing

amount of citric acid produced was followed by decrasing pH. pH of substrate became constant when amount of citric acid produced declined.

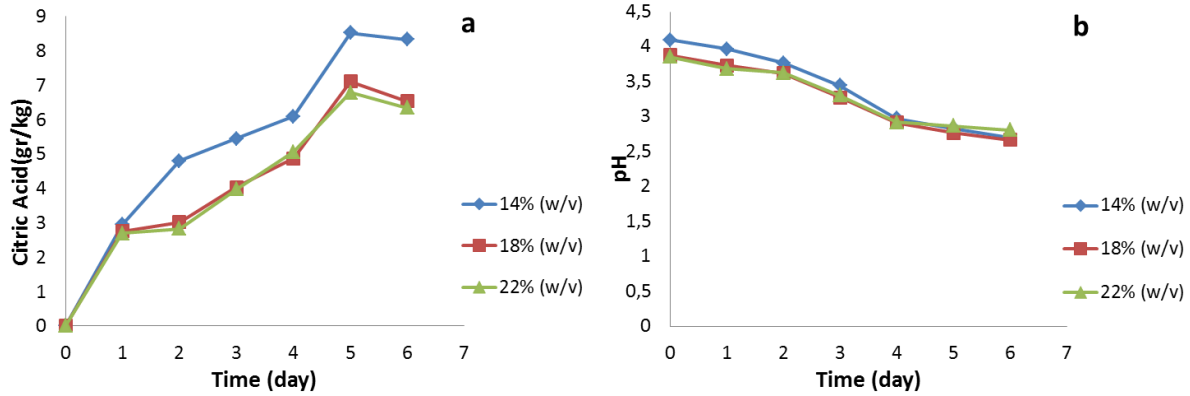

Figure 2. (a) Citric Acid Production; (b) Changes of pH
Environmental Condition : temperature of incubation 30°C, time of incubation 192h, inoculum 2x10^7 spora/mL, initial moisture level 70%, methanol 4% (v/w), without agitation

According Peksel and Kubicek (2001), citric acid accumulation takes place at a high concentration of carbon source. The use of sugar in the substrate (Figure 3a) was directly correlated to the production of citric acid. Citric acid was the primary metabolite of glucose metabolism. Therefore, the sugar residue found on the substrate has declined due to the use of sugar by *A.niger* in metabolism to produce citric acid. According to Torres *et. al.* (1996), regulation of citric acid accumulation was strongly influenced by glucose transport and phosphorylation of glucose. Glucose transport processes occured with the addition of 14% sucrose (w/v) was more optimal than the others so it produced larger quantity of citric acid.

Consumption of sugar in the substrate was strongly influenced by biomass of *A.niger* represented by extracellular enzyme activity (Figure 3b). Extracellular enzyme activity increased although fluctuations could occur at some points. The increase caused a greater consumption of sugar, so citric acid produced also increased every day. On the variation of 14% sucrose (w/v), extracellular enzyme activity increased substantially in the fifth day of fermentation, followed by reduction of the sugar residue. Presumably it underlied the formation of citric acid in large quantity.

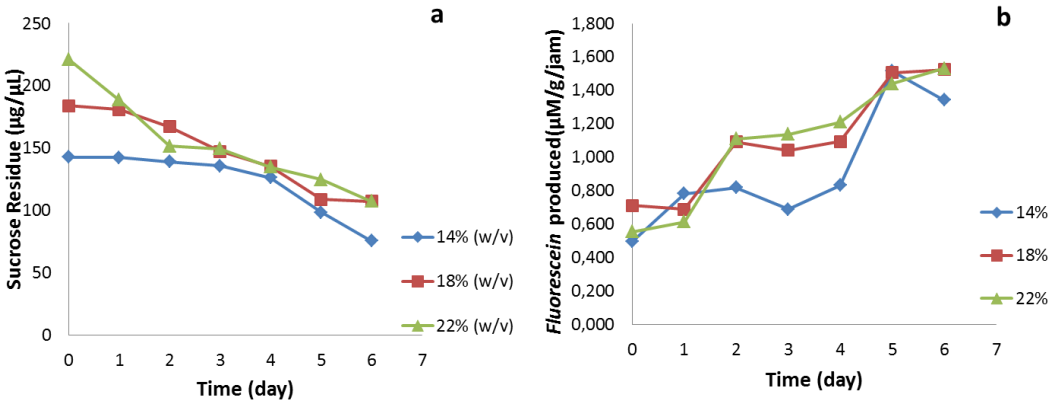

Figure 3. (a) Sucrose Residue on the medium; (b) Extracellular Enzyme Activity of *A. niger*
Environmental Condition : temperature of incubation 30°C, time of incubation 192h, inoculum 2x10^7 spora/mL, initial moisture level 70%, methanol 4% (v/w), without agitation

Solid State Fermentation of Pineapple Peels With The Variation of Ammonium Nitrate

Substrate added with sucrose 14% (w/v) and three variations of ammonium nitrate produced higher amount of citric acid. The optimum production of citric acid reached on the fourth day of fermentation, one day faster than previous fermentation (Figure 4). The optimum production of citric acid produced on the addition of ammonium nitrate 0.1 g/L, 0.25 g/L, and 0.4 g/L were 10.752, 10.624, and 11.136 g/kg, respectively.

Choe *et al.* (1991) and Yigitoglu et al. (1992) in Papagianni *et. al.* (2005) reported that addition of NH_4^+ ions during fermentation of citric acid would stimulate the rate of formation of citric acid and provided a consistent influence on PFK1 (phosphofructokinase 1 enzyme). The presence of NH_4^+ ions could stimulate the activity of PFK1 which played very important role in determining rate of glycolysis. This led to efficient citric acid production compare with condition when only sucrose added to the substrate.

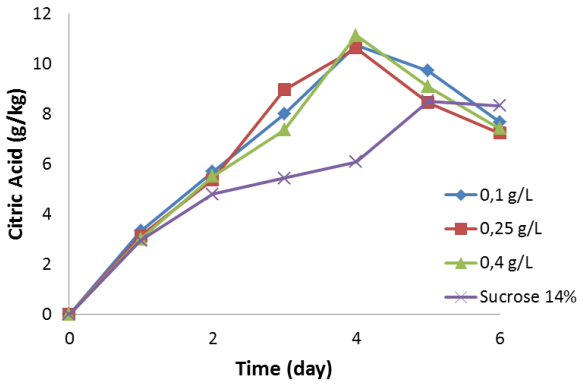

Figure 4. Citric Acid Production on Solid State Fermentation of Pineapple Peel with Variation of Ammonium Nitrate
Environmental Condition : temperature of incubation 30°C, time of incubation 192h, inoculum $2x10^7$ spora/mL, initial moisture level 70%, methanol 4% (v/w), without agitation

According to Wang et al. (1979 in Hadi, 1985), microorganism consume ammonium salt to form a mass of cells in the form of R-NH_3 with R groups is a carbon skeleton. It was shown that the addition of ammonium nitrate could increase biomass of *A.niger* (Figure 5).

Extracellular enzyme activity of *A. niger* grown on the substrate with the addition of ammonium nitrate higher than on a substrate that was given by sucrose only. Extracellular enzyme activity was correlated to the use of sucrose (Figure 6).

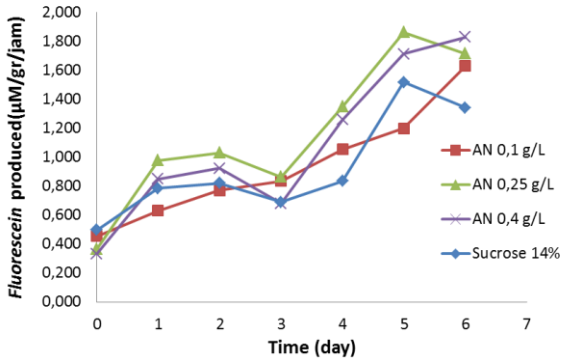

Figure 5. Extracelullar Enzyme Activity of *A. niger*
Environmental Condition : temperature of incubation 30°C, time of incubation 192h, inoculum $2x10^7$ spora/mL, initial moisture level 70%, methanol 4% (v/w), without agitation

Biomass of *A. niger* grown in the substrate with the addition of ammonium nitrate was higher than substrate added by sucrose only, as shown by less sucrose residue. As previously mentioned, less sucrose residue indicated more glucose entered the cell and more citric acid produced.When the amount of citric acid began to decline, extracellular enzyme activity would continue to increase. The citric acid was used as a carbon source for cell growth (Hadi, 1985).

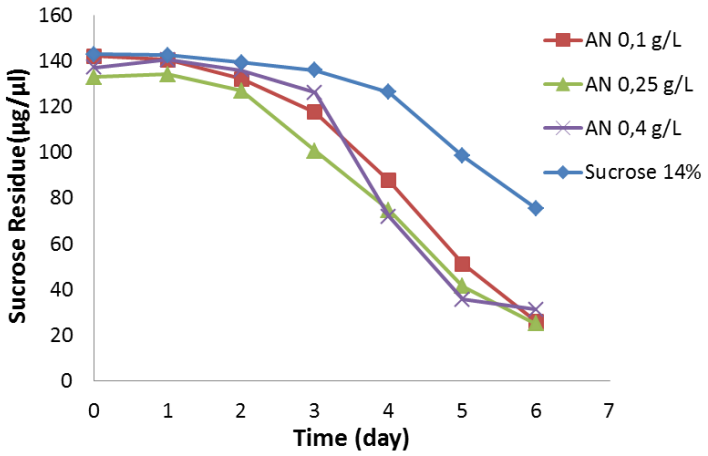

Figure 6. Sucrose Residue on the Medium
Environmental Condition : temperature of incubation 30°C, time of incubation 192h, inoculum 2x10^7 spora/mL, initial moisture level 70%, methanol 4% (v/w), without agitation

Figure 7 showed that the decrease in pH in the substrate given by ammonium nitrate were lower than those given by sucrose only. According to Reed *et al.* (1973 in Hadi, 1985), each binding of NH_3^+, H^+ ions will enter the environment, so that during the fermentation process H^+ ions in the fermentation medium will increase continuesly. Citric acid accumulation can be enhanced by creating conditions that are not suitable for aconitase enzyme by lowering the pH of the substrate below pH level suitable for the enzyme activity (Hadi, 1985)

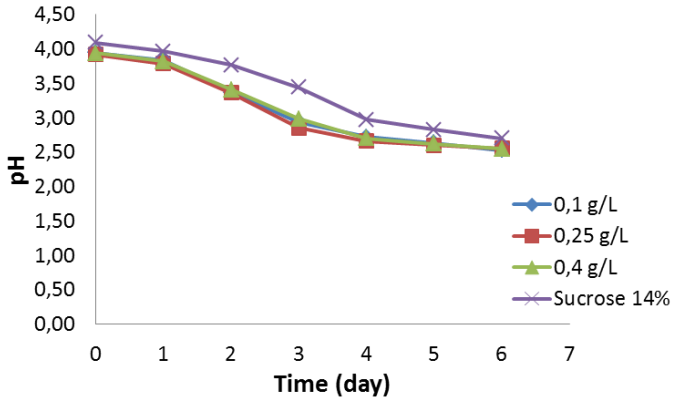

Figure 7. The Changes of pH
Environmental Condition : temperature of incubation 30°C, time of incubation 192h, inoculum 2x10^7 spora/mL, initial moisture level 70%, methanol 4% (v/w), without agitation

Conclusion

Based on this study, it can be concluded that pineapple peel can be used as a substrate to produce citric acid by *Aspergillus niger*. Citric acid produced from the fermentation using pineapple peel was 4.48 g/kg with a yield (the product of the substrate) of 9.180%.

Addition of carbon and nitrogen sources increased production of citric acid and yield (product of the substrate). The addition of sucrose 14% (w/v) produced highest amount of citric acid yield, 8.512 g / kg with a yield of 27.46% on the fifth day of fermentation.

Combination of 14% sucrose (w/v) and 0.1 g/L ammonium nitrate was capable of producing citric acid with a higher value, 10.752 g/kg with a yield of 28.10% on the fourth fermentation

References

Berovic M., Legisa, M. Citric Acid Production. *Biotechnology Annual Review* 13: 303–343, 2007.

Hadi, Y. *Pengaruh substitusi dedak oleh urea dan penambahan ampas tahu terhadap produksi asam sitrat secara fermentasi medium padat*. [Skripsi]. Bogor : Teknologi Pangan dan Gizi, Fakultas Teknologi Pertanian, Institut Pertanian Bogor, 1985.

Hadiati, S. Nanas Komersial Berdaun Tanpa Duri [Online]. Tersedia: http://pustaka.litbang.deptan.go.id [1 September 2013]

Kubicek, C.P. The Role of Sugar Uptake and Chanelling for Citric Acid Accumulation by *Aspergillus niger*. *Food. Technology and Biotechnology* 36(3): 173-175, 1998.

Papagianni, M., Wayman, F., Mattey, M. Fate and Role of Ammonium Ions During Fermentation of Citric Acid by *Aspergillus niger*. *Applied and Environmental Microbiology* 71(11): 7178–7186, 2005.

Peksel, A., Kubicek, C.P. Effect of Sucrose Concentration During Citric Acid Accumulation by *Aspergillus niger*. *Turkish Journal of Chemistry* 27: 581-590, 2001.

Soccol, C.R., Vandenberghe, L.P.S., Rodrigues, C., Pandey, A. New Perspectives for Citric Acid Production and Application. *Food Technology and Biotechnology* 44: 141-149, 2006.

Torres, N.V., Riol-Cimas, J.M., Wollschek, M., Kubicek, C.P. Glucose Transport by *Aspergillus niger*: The Low-Affinity Carrier is Only Formed During Growth on High Glucose Concentrations. *Applied Microbiology and Biotechnology* 44:790-794, 1996.

FEED - 05

The Optimizing of Growth and Quality of *Chroococcus sp.* as ASUH feed supplement for broiler

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ABSTRACT - Microalgae has potentials as element of ration or natural ASUH (safe, healthy, whole and halal) feed supplement for it contains nutrition and active component, decreases cholesterol level and resulting darker yolk. *Chroococcus sp* is type of blue alga (*Cyanophyceae*) which, its economical potential need to be revealed. In terms of mass production, it is important to find correct, cheap and easy to feed nutrition for breeders. The objective of the research is to find out the information to optimize growth and quality of *Chroococcus* sp as ASUH feed supplement for broiler. Test using sedgwick rafter method conducted to find out the optimizing of growth and quality of *Chroococcus* sp while AOAC method applied to test quality of its nutrition. The result shows that *Chroococcus* sp grew well at technical medium Phyto-s with k relative 0.9866, crude protein 39.871%, fat 2.425%, b Carotene 7.43 mg/gram, Vitamin C 3.01 mg/kg and vitamin E 1.05%. It is concluded that *Chroococcus sp* potential to be natural and ASUH feed supplement and Phyto-s can be used as nutrition for mass production.

Keyword : ASUH, *Chroococcus* sp, feed suplement, phyto-s

FULL PAPER WITHDRAWED by AUTHOR

FEED - 06

Early Development of Nypa Palm Worm *Namalycastis rhodochorde* (Polychaeta, Nereididae): Biological Perspective for Mass Production

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ABSTRACT - Nereididae worms including *Namalycastis* have been considered as potential broodstock nutrition in aquaculture industry. Meanwhile, *Namalycastis* intensive culture itself is still facing problems in mass production due to limited information on reproduction especially in fertilization and production of larvae and juveniles. The purpose of this research was to find optimum condition for fertilisation and production of Nypa palm worm larvae (*Namalycatis rhodochorde*). Gamete samples were collected using a tube glass capilarry injected to ventro-lateral part of body segment of matured worm. Artificial fertilization was done by mixing the sperms and oocytes in same container with sterillized sea water as the media. The initial development process prior to fertilisation was observed until benthic phase larvae (3-setigers). Fertilization was done under salinity of 7-21‰ and water temperature of 25-29°C. The cleavage was achieved within 15-25 minutes and larval stage within 72-80 hours after fertilisation. The fertilization and larval development of *N. rhodochorde* were highly influenced by the water salinity and temperature, room temperature and culture media.

Keywords: *aquaculture, baits, development, larval, Namalycastis rhodochorde, Polychaeta*

FULL PAPER ACCEPTED by HAYATI JOURNAL

FEED - 07

Studies on *Vibrio* Population in Zero Water Discharge System through *Chlorella* sp., Nitrifiying Bacteria and Probiotics Bacteria (*Bacillus megaterium* and/or *Bacillus amyloliquefaciens*) Addition for Nursery Phase of *Macrobrachium rosenbergii* De Mann

Pingkan Aditiawati[1,2], Puspasari Widyaranti[1], Dea Indriani Astuti[1], Gede Suantika[1]& Hanslibrery Simanjuntak[2]

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ABSTRACT - The aim of this study was testing the effectiveness of zero-water discharge systems ~~with~~ combined with probiotic bacteria *Bacillus megaterium* and/or *Bacillus amyloliquefaciens*, to decrease *Vibrio* population. In addition, this research would also include survival rate and biomass production of giant river prawn. This research was conducted in 2 experimental treatments using 5 outdoor cemented ponds detailed as 1 control ponds (C), 2 treatment I ponds (T1) and 2 treatment II ponds (T2) The highest density of detected *Vibrio* during prawn culture period in control (C), treatment I (T1), and treatment II (T2) ponds were 9.4×10^2; $9.8 \times 10^2 \pm 1.03 \times 10^3$; and $4.73 \times 10^3 \pm 3.54 \times 10^2$ which happened during the first week of culture period. Despite of high *Vibrio* number in treatment 2, based on the results of the ANOVA statistical test performed, this value was not significant compared to other treatments ($p = 0.03$; $\alpha = 0{,}05$). The best survival rate was found at treatment I (*Chlorella* sp. 10^4 cells /ml + 10^5 nitrifying bacteria cells/ml + *Bacillus megaterium* 10^4cfu/ml), followed by treatment II (*Chlorella* sp. 10^4 cells /ml + nitrifying bacteria 10^5 cells/ml + *Bacillus megaterium* 10^4cfu/ml and *Bacillus amiloliquefaciens* 10^4cfu/ml) and the last was control ponds, with survival rate given (based on the sequence above) are 58, 28%, 52,36%, and 39,77%. Based on these results, addition of probiotic bacteria B. *megaterium* 10^4cfu/ml able to decrease *Vibrio* growth in zero-water discharce system and increase giant river prawns productivity.

Keywords: *Bacillus amyloliquefacien; Bacillus megaterium; probiotic; Vibrio; zero-water discharge*

FULL PAPER WITHDRAWED by AUTHOR

The Nutritive Performances of PUFA- Concentrate Supplemented with Yeast and *Curcuma xanthorrhiza*, Roxb Stored in 2-6 weeks

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ABSTRACT - Concentrate containing fatty acid sources, roasted corn grain, roasted soy bean meal, and corn oil, designated as PUFA- concentrate for dairy goat. There were four concentrates, no supplement (Y0C0), 5g yeast (Y5C0), 20g curcuma powder (Y0C20), and 5g yeast with 20g curcuma powder (Y5C20). These concentrates were evaluated for nutrition and fatty acid contents during 2 and 6 weeks of storage. Results showed that based on the contents of dry matter (DM), organic matter (OM), ether extract (EE), crude protein (CP), N- free extract (NFE), gross energy (GE), acid detergent fiber (ADF), Ca, P, and *Saccharomyces cereviseae* (2.4×10^7 cfu/g) were significantly ($p<0.05$) remained stable as caused by unchained moisture of PUFA- concentrate with combined supplements in the 6 weeks of storage. The total PUFA (P), P/S, Monounsaturated fatty acid (MUFA), and long chained fatty acid contents were found higher in PUFA- concentrate with 20g curcuma powder. Whereas, the PUFA- concentrate with 5g yeast and 20g curcuma powder was higher in unsaturated (U) fat and the ratio of U/S. Combining all nutrient performances during the storage of 2-6 weeks, the PUFA- concentrate with 5g yeast and 20g curcuma powder was considered nutritionally healthy.

Keywords: curcuma powder, fatty acid, PUFA-concentrate, yeast.

FULL PAPER WITHDRAWED by AUTHOR

Chemical Composition and Nutrient Quality of Swamp Forage Ensiled with *Lactobacillus plantarum*

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ABSTRACT - Availability of forage for ruminants, especially during drought season is not always caused by lower production, but may caused by inability to managed forage at heavy production period during rainy season. A study was designed to evaluate an alternative for this problem. The research was conducted in Basic Science Laboratory of Agriculture college at UNISKA in Banjarmasin. Materials used were swamp forage (SF), rice bran, inoculants of *Lactobacillus plantarum* 1A-2, L. *plantarum* 1BL-2, molasse, and plastic silos. The experimental design used was completely randomized with 3 treatments and 4 replications, there were 12 experimental units. S1 = SF + *L. plantarum*, (S2) : SF+ *L. plantarum* 1BL–2, and (S3) = SF + molases. Results showed that silage with Plantarum 1BL-2 was considered good quality with pH (3.84), Total LAB or Lacto acid bacteria (7.8 x 10^8cfu/ml), NH_3 (90.12g N/kg), VFA (108.72mM), WSC (2.83% DM), dry matter (DM) digestibility (63.21%), Organic matter(OM) digestibility (62.32%). Quality of nutrition showed DM (25.95%), protein (13,96%), crude fiber (14.89%), ether extract (8.42%), and ash (7.72%). Score of the silage was 88.23, which was falling within the range of 80-100, considered good quality of silage.
Keywords: silage, *Lactobacillus plantarum*, swamp forage, molases

FULL PAPER WITHDRAWED by AUTHOR

Effects of Municipal Compost from TPK Sarimukti, Cipatat, on Vegetative Growth of Chilli (*Capsicum annuum* L.), and on Soil Quality

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ABSTRACT – Sarimukti landfill, Cipatat, Bandung, is a temporary landfill to replace Leuwigajah landfill since 2006. In this landfill, there is a Sarimukti's composting area, where organic municipal wastes are converted into composts. However, the effect of Sarimukti's compost into environments & living things, such as plants, has not been studied further. The aims of this research were (1) to evaluate the effect of compost on vegetative growth of chilli (*Capsicum annuum* L.) and (2) to evaluate the effect of compost on soil quality. Experiments were carried out by the method of randomized block design (RBD) with two kinds of control, 100% soil and 100% compost as positive and negative controls, as well as the media treatment of a mixture of compost and soil on the ratio (1:1), (1:3) and (1:5). Growing media (1:1) gave the best effect on the growth of chilli. Also, in general, soil quality, such as, physical and chemical properties of soil showed an increase in the presence of compost in the growing media.

Keywords: compost, Sarimukti, growth, vegetative, chilli, soil

The Role of Leaf Extracts as Plant-activator in Enhancing Tomato Plant Growth, Productivity and Resistance to CMV (Cucumber Mosaic Virus)

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ABSTRACT -- Tomato (*Lycopersicon esculentum* Mill.) is one of horticultural crops which has a high economic value, but its production may decline due to the disease, such as Cucumber Mosaic Virus (CMV). There were several ways to improve its resistance, growth, and fruit productivity from the disease during planting process, such as by the addition of plant-activator derived from plant extracts. The aim of this study were to evaluate effect of CMV infection on the growth of tomato plants, as well as effect of leaf extracts of Japanese Glorybower (*Clerodendrum japonicum*) and Madagascar Periwinkle (*Catharanthus roseus*) to increase resistance against viruses, growth, and fruit productivity. The study was conducted using a randomized block design with factorial treatment. The first factor was cultivar of tomato plants (*Lycopersicon esculentum* Miller cv. Intan and cv. CL 6064). The second factor was plant leaf extracts: *C. japonicum* and *C. roseus*, each with the level of 50% (w/v) concentration. Each treatment was repeated three times, and each repeated group consisted of five individual tomato plants. The results of experiment showed that level of plant resistance, growth, and fruit productivity of tomato plants were improved after application of the plant extracts. In tomato plant cv. Intan, leaf extract of *C. japonicum* was the most potential extract which could improve plant resistance, growth, and fruit productivity. In cv. CL 6064, leaf extract of *C. japonicum* was the most potential extract which could improve plant resistance and growth, while enhancement of fruit productivity was related to the application of *C. roseus* plant leaf extract.

Keywords: CMV, fruit productivity, growth, plant extract, tomato

FERTILIZER - 03

Isolation and Identification of Tomato (*Solanum lycopersicum*) MOL (Indigenous Microorganism) as Basic Development for Multifunctional Biofertilizer

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ABSTRACT – The use of microorganisms as biological agents have been widely proven as a way to increase crop productivity. Solution of MOL (indigenous microorganism), batch fermentation of local organic waste of an area, is one of the sources of biofertilizer that have been used in Indonesia as a substitute for organic fertilizer to increase plant productivity but still have not been studied scientifically. Most biofertilizer products consist of single microorganism with specific function such as N_2 fixing bacteria. Meanwhile MOL consists of a mixture of microorganisms, so it is expected to contain heterotrophic microorganisms and microorganisms with specific roles for plants such as phosphate-solubilizing and nitrogen-fixing bacteria and functioned as multifunctional biofertilizer. In this study, microorganisms from tomato-based MOL solution (*Solanum lycopersicum*) will be isolated and availability of N_2-fixing bacteria, phosphate-solubilizing bacteria, and plant growth promoting bacteria will be analyzed. MOL solution used was the result of fermentation of tomatoes, coconut water, and brown sugar which incubated for 15 days with stirring every 3 days. This study found 4 bacterial isolates that is known to have capabilities as plant growth promoter, 1 isolate of N-fixing, and 1 isolate of P-solubilizing bacteria.

Keyword: MOL, N-fixer, P-solubilizer, PGP bacteria, identification.

FULL PAPER WITHDRAWED by AUTHOR

Effect of Fertilizer to Bee Visitation in Tomato (*Lycopersicum esculentum*)

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ABSTRACT - Tomato (*Lycopersicon esculentum* Mill.) is common commodity of Indonesia farming. However, monthly production is unstable due to lack of pollination services. Previous study indicated that utilization of two common local domesticated bees as pollination agent, local honey bee (*Apis cerana* L.) and stingless bee (*Trigona iridipennis*), may improve and stabilize local tomato production. However, since visitation rate of bees correlated with total amount of reward provide by flowers and successful fruit formation, it is necessary to understand whether the additional nutrition to plant effects visitation rate of bees. During this study, total visitation rate and total numbers of pollinated flowers by honey bee and stingless bee on plant received specific nutrition (High Nitrogen, High Phosphor, and no fertilizer) measured. Total fruit production, average weight and size also measured in order to correlated pollination efficiency with visitation rate. Result of this research showed that Phosphor stimulate highest flower production while encourage high visitation rate from both bees species tested ($P<0.05$) which in advance improve quantity and quality of fruit produced ($P<0.05$). Based on the results, it is concluded that farmer should regulated their fertilizer to concord the plant need, especially during critical period of development and biomass production.

Keywords: Fertilizer, flower, honey bee, pollination, stingless bee, tomato, visitation rate.

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is major agricultural commodity of local farming in Indonesia, especially West Java. Even though large area of agriculture is dedicated for tomato plantation, total number of production and quality of tomato produced are unstable. This situation might caused by lack of pollination in local tomato plantation (Putra, in press). Tomato flowers are self-compatible and wind pollinated when planted in open field (Free, 1993). However, in order to produce best fruit they need a pollination agent to vibrate the anthers and release the pollen and create perfect pollination (Banda and Paxton, 1991). On the other hand, this agent also allow farm to produce synchronize fruit production to gain more profit.

Naturally, wild insects around agriculture area would act as pollination agents (Ricketts, 2004; Tscharntke *et. al.*, 2005; Greenleaf and Kremen, 2006; Klein *et. al.*, 2007; Kremen and Chaplin-Kramer, 2007; Winfree *et. al.*, 2008). However, activities of intensified farming system like the use of synthetic pesticide, lost of food plants, invasive species etc. caused rapid declines of wild insect pollinator (Buchmann and Nabhan, 1996; Kearns *et. al.*, 1998; Biesmeijer *et. al.*, 2006; Klein *et. al.*, 2008). This situation led to decreasing agriculture yield due lack of pollination (Kevan and Phillips, 2001). In order to overcome this problem, honey bees usually apply as pollinator agent.

Previous study showed that local honey bee (*A. cerana*) and stingless bee (*T. iridipennis*) may act as pollination agent of tomato (Putra, in press). However, that study also found that difference in nutrition received by tomato masked the benefit of these bees on fruit production. Additional nutrition received by plant provide plant with extra resource for flowers, fruit, and seed production (Campbell and Halama, 1993), to reduce fruit abortion (Stephenson, 1981), to increase size of flowers (Campbell and Halama, 1993; Parson *et. al.*, 1995; Nagy and Proctor, 1997; Wyka and Galen, 2000; Heer and Komer, 2002), and influence pollinator visitation rate (Galen, 1985; Galen *et. al.*, 1987; Schemske and Horvitz, 1988; Johnston, 1991; Heer and Komer, 2002). However, most of these studies were conducted in wild plant not in cultivated plants. Thus, questions on do local tomato really need pollinator agent or just more fertilizer remains unanswered. This research tries to answer this question which in advance would help local farmer to maintain their farm in order to increase and maintain high tomato production.

Materials and Methods

Study Area

The pollination experiment was conducted at local farm in North Bandung, West Java, Indonesia from March to August 2012. Average daily temperature of study site was 18-25^{o}C with humidity 60-75%.

Materials

Fertilizer

During this study, commercial synthetic fertilizer that commonly used by local farmers was used.

Tomatoes

Each plant was planted on medium with 40% soil, 30% sand and 30% compost. Plants selected for this research were free of pest with average height of 50 cm. During this study, 30 tomato plants arranged in 3 rows of 10 plants each, with wide aisle (about 2 meters) between the rows.

Bees

Three colonies (≈1500 bees per colony) of *T. iridipennis* and three colonies of *A. cerana* (≈10,000 bees) were introduced into farm. All colonies originated from wild colonies found at area surrounding farm, kept in bee hive made from wood and acclimatize for 3 months prior to study.

Methods

Fertilizer Application

To assess the effects of N and P addition to *L. esculentum*, we conducted a manipulative field experiment. Thirty plants of comparable size, each separated by at least two m from one another, were selected. Plants were assigned to one of two treatments, each with 10 replicates: (1) Control (no N or P added only agricultural soil), (2) + Nitrogen (N added), and (3) + Phosphor (P added). We applied 30 g of N in the form of urea-N pellets (NPK 40-0-0) dissolved in 2 L of water while application of P was using 30 g P_2O_5 dissolved in 2 L water. All fertilizers were applied once per week.

Flowering condition

During this study, we noted effect of addition fertilizer to flowering time and flower number. This observation was conducted from the beginning of first flowering until last flower senescence.

Bee visitation frequency

Frequency of bee visitation was observed during flowering period based on method developed by Klein *et al.* (2003). Observation conducted only at sunny day between 0700 and 1600 (local time). Observation conducted 5 minutes per hour for three consecutive days at different plant. Total number of flowers observed was 100 and total visitation frequency calculated by polled all data obtained from three days observation.

In simultaneous time, another observer recorded amount of time spent by individual bees in one flower and defined as handling time.

Bee pollination efficiency

For this experiments, 10 flowers, that still not bloomed, in each of all plants used were randomly selected and tagged. Each group of flower was bagged with mesh nylon bag (diameter 1 mm). Glue were applied at the twig were flowers located to prevent ants from entering flower. Bags were removed when flowers started to bloom. Observation for bee pollination efficiency started from removal of the bag until bee transferred pollen to female flower by bee. After pollination process, flowers were bagged until fruit was produced. This group of treatments stated as honey bee (HB) and stingless bee (SB) group.

Pollination efficiency were measured by

$$\text{Pollination efficiency} = \frac{\text{Total numbers of flowers that produce fruits}}{\text{Total numbers of observed flowers}}$$

Statistical Analysis

We used Tukey's mean separation test to find any difference of fertilizer effect on average number of flower produced, flowering time, bee visitation rate, and pollination efficiency. Significant value for this test is $P<0.05$. All tests were conducted by statistical program STATISTICA 6.

Result and Discussion

Flowering condition

During this study we found that additional fertilizer did not change first flowering time (Tukey's mean separation test, $P>0.05$) while plant pollinated with high phosphor produced more flowers (Tukey's mean separation test, $P<0.05$) (Figure 1). Our finding agrees with Shuel (1957) and Wang *et al.* (2011) that additional phosphor may increase total number of flowers produced significantly. However, this study showed that additional phosphor did not hasten early flower production, although

it reduced time needed to achieve peak flowering period. It seems there are other factors that worked on stimulating flowering than high phosphor content on soil.

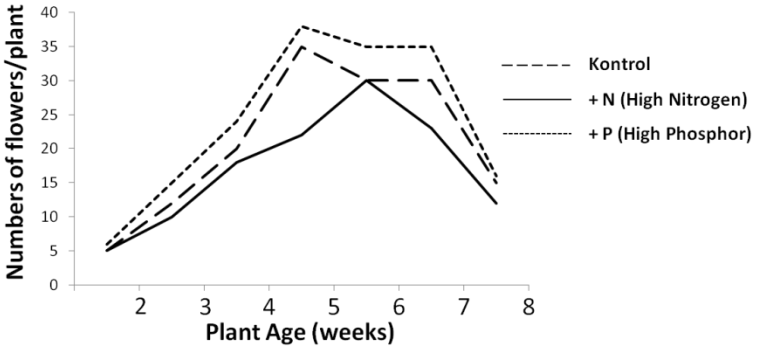

Figure 1 Average number of flowers per week produced by tomato plant with different application of chemical fertilizer

In this research we also found that plant grow on control soil produced similar number of flowers with plant fertilized with high phosphor. This result may indicate that local agriculture soil already contained enough phosphor probably due to heavy fertilizer application which in common practices of local farmer.

Bee Visitation Rate

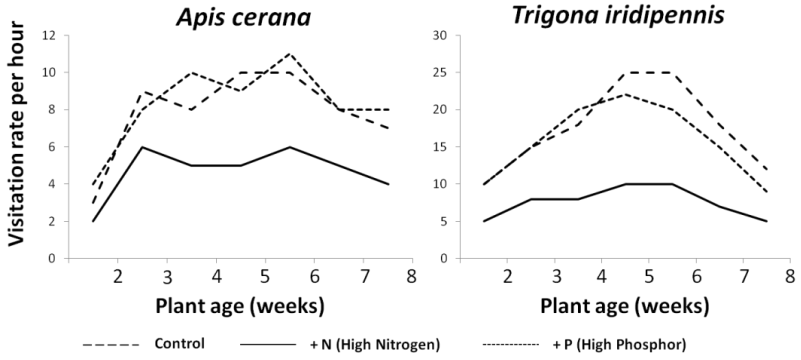

Figure 2 Average number of flowers per week produced by tomato plant with different application of chemical fertilizer

Both *A. cerana* and *T. iridipennis* had higher visitation rate at plant grow on control soil and soil fertilized with high phosphor (Figure 2) while *T. iridipennis* had higher visitation rate than *A. cerana*. This result probably caused by bigger floral display due to higher numbers of flowers (Ohashi & Yahara, 1998) and distribution of reward for bees (Lefebre & Piere, 2006) as plant fertilized with high nitrogen produced less flowers.

Bee Pollination Efficiency

Visitation rate influence quantity and quality of fruits produced. This study showed that visitation by *A. cerana* produced higher number of fruit per plant than *T. iridipennis* (Table 1). This probably caused due to ability of *A. cerana* to release pollen from anther earlier than *T. iridipennis* that induce pollination and fruit production (Putra, in press). On the other hand, high visitation by *T. iridipennis* produced heavier and bigger tomato which is probably due to higher number pollen deposited on stigma (Serrano and Guerra-Sanz, 2006). This finding also showed that production of fruit is not only

influence by fertilizer but also by pollination success and specific type of fertilizer is needed to ensure better production.

Table 1 Number, weight, and diameter of fruit pollinated by *Apis cerana* and *T. iridipennis*

Treatment	Variables	Pollination agent	
		Apis cerana	*Trigona iridipennis*
Control	Number of fruits per plant	23 ± 5.6	20 ± 3.1
	Weight (gram)	23.54 ± 5.93	25.53 ± 5.51
	Diameter (mm)	14.43 ± 6.51	18.59 ± 6.07
High Phosphor	Number of fruits per plant	25 ± 4.6*	18 ± 2.1
	Weight (gram)	25.51 ± 9.93	29.53 ± 7.51*
	Diameter (mm)	15.53 ± 7.51	19.59 ± 4.07*
High Nitrogen	Number of fruits per plant	15 ± 4.6	13 ± 3.1
	Weight (gram)	23.21 ± 9.93	24.45 ± 4.41
	Diameter (mm)	13.43 ± 7.51	17.29 ± 2.07

*) indicated significant value tested by Tukey's mean separation test with significant value $P<0.05$

Conclusion

Phosphor increased the number of flowers produced that correlated with higher reward for pollinating bees. Higher number of flower increased visitation rate that improve the quantity and quality of tomato produced. Specific type of fertilizer is needed in order to improve fruit production and quality. Further research is needed to find function of each type of fertilizer to specific period of local plant cultivar development.

Acknowledgement

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References

Banda, H.J., Paxton, R.J. Pollination of Greenhouse Tomatoes by Bees. *Acta Horticulturae* 288: 194-198, 1991.

Biesmeijer, J.C., Roberts, S.P.M, Reemer, M., Ohlemüller, R., Edwards, M., Peeters, T. Schaffers, A.P., Potts, S.G., Kleukers, R., Thomas, C.D., Settele, J., Kunin, W.E. 2006. Paralel Declines in Pollinators and Insect-Pollinated Plants in Britain and the Netherlands. *Science* 313: 351-354, 2006.

Buchmann, S.L., Nabhan, G.P., *The Forgotten Pollinators*, Island Press, Washington, D.C. USA, 1996.

Campbell, D.R., Halama, K.J. Resource and Pollen Limitations to Lifetime Seed Production in a Natural Plant Population. *Ecology* 74: 1043-1051, 1993.

Free, J.B. *Insect Pollination of Crops*, 2nd ed., Academic Press, Harcourt Brace Javanovich Publishers, 1993.

Galen, C. Regulation of Seed Set in *Polemonium viscossum*: Floral Scents, Pollination, and Resources. *Ecology* 66: 792-797, 1985.

Galen, C., Zimmer, K.A., Newport, M.E. Pollination in Floral Scent Morphs of *Polemonium viscossum:* A Mechanism for Disruptive Selection on Flower Size. Evolution 41: 599-606, 1987.

Greenleaf, S.S., Kremen, C. Wild Bee Species Increase Tomato Production but Respond Differently to Surrounding Land Use in Northern California. *Biology Conservation* 133: 81-87, 2006.

Heer, C., Korner, C. High Elevation Pioneer Plants are Sensitive to Mineral Nutrient Addition. *Basic Applied Ecology* 3: 39-47, 2002.

Johnston, M.O. Natural Selection of Floral Traits in Two Species of Labelia with Different Pollinators. *Evolution* 45: 1468-1479, 1991.

Kearns, C.A., Inouye, D.W., Waser, N.M. Endagered Mutualisms: The Conservation of Plant-Pollinator Interactions. *Annual Review of Ecology and Systematic* 29: 83-112, 1998.

Kevan, P.G., Philips, *T.P*. The Economic Impacts of Pollinator Declines: An Approach to Assessing the Consequences. *Conservation Ecology* 5: U211-U230, 2001.

Klein, A.M, Vaissiere, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C., Tscharntke, T. Importance of Pollinators in Changing Landscapes for World Crops. *Proceeding of the Royal Society Series B: Biological Sciences* 274: 303–313, 2007.

Klein, A.M., Cunningham, S.A., M. Bos, M., Steffan-Dewenter, I. Advances in Pollination Ecology from Tropical Plantation Crops. *Ecology* 89: 935-943, 2008.

Klein, A.-M., Steffan-Dewenter, I, Tscharntke, T. Fruit Set of Highland Coffee Increases with the Diversity of Pollinating Bees. *Proceeding of the Royal Society of London, Series B: Biological Sciences* 270: 955-961, 2003.

Kremen, C., Chaplin-Kramer, R. Insects as Providers of Ecosystem Services: Crop Pollination and Pest Control. Insect Conservation Biology in *Proceedings of the Royal Entomological Society's 23rd Symposium* (eds. Stewart, A.J.A., T.R. New and O.T. Lewis), pp. 349-382,CABI Publishing, Wallington, UK, 2007.

Lefebre, D., Piere, J. Spatial Distribution of Bumblebees Foraging on Two Cultivars on Tomato in a Commercial Greenhouse. *Journal of Economic Entomology* 99: 1571-1578, 2006.

Lehtila, K., Strauss, S.Y. *Leaf Damage by Herbivores Affect Attractiveness to Pollinators in Wild Radish Raphanius raphaniustrum*. Oecologia 111: 396-403, 1997.

Nagy, L., Proctor, J. Plant Growth and Reproduction on a Toxic Alpine Ultramfic Soil: Adaptation to Nutrient Limitation. *New Phytology* 137: 267-274, 1997.

Ohashi, K., Yahara, T. Effects of Variation in Flower Number on Pollinator Visits in *Cirsium purpuratum* (*Asteraceae*). *American Journal of Botany* 85: 219-224, 1998.

Parsons, A.N., Press, M.C., Wookey, P.A., Robinson, C.H., Callaghan, T.V., Lee, J.A. Growth Responses of *Calamagrotis lapponica* to Stimulated Environmental Change in the Sub-Arctic. *Oikos* 72: 61-66, 1995.

Ricketts, T.H. Tropical Forest Fragments Enhance Pollinator Activity in Nearby Coffee Crops. *Conservation Biology* 18: 1262-1271, 2004.

Schemske, D.W., Horvitz, C. Plant-Animal Interactions and Fruit Production in a Neotropical Herb: a Path Analysis. *Ecology* 69: 1128-1137, 1988.

Serrano, A.R., Guerra-Sanz, J. Quality Fruit Improvement in Sweet Pepper Culture by Bumblebee Pollination. *Scientia Horticulturae* 110: 160-166, 2006.

Shuel, R.W. Some Aspects of the Relation Between Nectar Secretion and Nitrogen, Phosphorus, and Potassium Nutrition. *Canadian Journal of Plant Science* 37: 220-223, 1957.

Stephenson, A.G. Flower and Fruit Abortion: Proximate Causes and Ultimate Functions. *Annual Review of Ecology and Systematic* 12: 253-279, 1981.

Tscharntke, T., Klein, A.M., Kruess, A., Steffan-Dewenter, I., Thies, C. Landscape Perspectives on Agricultural Intensification and Biodiversity-Ecosystem Service Management. *Ecological Letter* 8: 857-874, 2005.

Wang, S., Jing, J., Li, T., Li, H., Wang, C., Wang, Y., Liu, G. Influence of Nitrogen, Phosphorus, and Potassium Fertilization on Flowering and Expression of Flowering-Associated Genes in White Birch (*Betula platyphylla* Suk.), Plant Molecular Biology Reporter 29: 794-801, 2011.

Winfree, R., Williams, N.M., Gaines, H., Ascher, J.S., Kremen, C. Wild Bee Pollinators Provide the Majority of Crop Visitation Across Land-Use Gradients in New Jersey and Pennsylvania, USA. *Journal of Applied Ecology* 45: 793-802, 2008.

Wyka, T., Galen, C. Current and Future Costs of Reproduction in Oxytropis sericea, a Perennial Plant from the Colorado Rocky Mountain, USA. *Arctic Antarctic and Alpine Research* 32: 438-448, 2000.

Effect of Silica Fillers on Characterization of Cellulose-Acrylamide Hydrogels Matrices as Controlled Release Fertilizers

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ABSTRACT - Controlled Release Fertilizers-based hydrogels was synthesized by graft copolymerization of acrylamide onto the rice straw cellulose backbones in the presence of silica fillers using simultaneous graft copolymerization by gamma irradiation as initiator. Evidence of the silica presence on grafted cellulose was obtained from FTIR. X-ray diffraction analysis showed that the crystallinity was reduced through silica fillers added. The effect of silica content on grafting efficiency, gel fraction, Young modulus, swelling degree, and urea loading were examined. It was found that grafting efficiency and gel fraction decrease with increasing silica added inversely with Young modulus. Water swelling and loading urea fertilizers were conducted and the results showed that swelling degree and urea fertilizers loading increased first and then decreased with increasing silica added. The effect of the silica fillers had implications in the mechanism of controlled release urea fertilizers that diffusion-controlled mechanism became dominant, which attributed to the decreasing of urea diffusion coefficient.

Keywords: cellulose hydrogels, controlled release fertilizers, gamma irradiation, graft copolymerization, silica fillers, urea diffusion

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FERTILIZER - 06

Isolation and Molecular Identification of Endophytic Bacteria from Rambutan Fruits (*Nephelium lappaceum* L.) Cultivar Binjai

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Abstract. Interactions between plants and endophytic bacteria are mutualistic. Plant provides nutrient for bacteria, and bacteria will protect the plant from pathogen, help the phytohormones synthesis and nitrogen fixation, and also increase absorption of minerals. These bacteria called plant growth-promoting bacteria (PGPB). The aim for this study is to identify endophytic bacteria on rambutan (*Nephelium lappaceum* L.) cultivar Binjai with 16S rRNA. Sequencing results showed that the bacteria is derived from genus *Corynebacterium, Bacillus, Chryseobacterium, Staphylococcus* and *Curtobacterium*, which suspected play a role as PGPB.

Keywords: 16S rRNA gene, endophytic bacteria, PGPB, rambutan

FULL PAPER ACCEPTED by HAYATI JOURNAL

A Preliminary Investigation; Phosphate Solubilizing Bacteria which Adaptive to Vinasse

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ABSTRACT - Microorganisms identified as Phosphate Solubilizing Bacteria (PSB) were successfully screened from sugarcane agricultural estate soil at Jatiroto which polluted vinasse. This investigation found that five differences isolates (pvk-5a, pvk-5b, pvk-6b, pvk-7a, and pvk-8a) were detected as PSB by screening on Pikovskaya Agar medium. Among of them, only three isolates (pvk-5a, pvk-5b and pvk-7a) can be grown and adaptive on vinasse based medium without any nutrients added. The tree isolates characterized as coccus and gram negative with no endospores detected. Suggested, these three isolates may be able to be used as biofertilizer agent to support organic farming.

Keywords: psb, vinasse, coccus, gram negative.

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FERTILIZER - 08

Synthesis and Properties of Controlled-Release Fertilizers based on Hydrogels of Chitosan-Acrylamide Graft Copolymers Using Gamma Irradiation

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ABSTRACT - Synthesis of Controlled Release Fertilizers (CRF) based chitosan-acrylamide graft copolymers hydrogels has been performed with radiation processing technology using Co-60 gamma irradiation. The evidence of grafting was obtained from comparison of FTIR of the chitosan and grafted chitosan. X-ray diffraction analysis showed that the crystallinity was reduced through graft copolymerization. The influence of radiation dose on grafting efficiency, gel fraction, crosslink density, swelling degree, urea loading and release were examined. Grafting efficiency and gel fraction of prepared hydrogel obtained optimum at the radiation dose of 15 kGy. Swelling degree and urea fertilizers loading decrease with increased radiation dose inversely with crosslink density. This type of urea fertilizers release mechanism of prepared hydrogels is found to be a non Fickian diffusion. The increasing radiation doses from 10 to 35 kGy decreases diffusion coefficient of urea fertilizers from the hydrogels from 1.076×10^{-7} to 6.104×10^{-9} cm^2/s. It may be concluded that by changing the crosslink density with radiation doses varying, a rate-controlled fertilizers release is obtained.

Keywords: chitosan hydrogels, controlled release fertilizers, gamma irradiation, graft copolymerization, radiation doses, urea diffusion

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FIBER - 01

Chemical Characterization of Poly-gamma-Glutamic Acid Produced by Bacterial Strain Isolated from Indonesian Natto

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ABSTRACT - A strain of bacteria nominated as *Bacillus* sp. strain-S have been successfully isolated from Natto produced in Cianjur, Indonesia. This strain was used to produce poly-gamma-glutamic acid (gamma-PGA) using liquid medium with citric acid, L-glutamic acid and ammonium sulfate as the main nutrients. Production of gamma-PGA in this medium is growth-associated with the highest production (3.17 $g.L^{-1}$) reached at 48 h of fermentation time. Characterization using FTIR spectroscopy identified that the material produced by *Bacillus* sp. strain-S has functional groups of carboxyl, hydroxyl, amide, and amine that highly correlate with functional groups that present in gamma-PGA. In addition, the results of NMR analysis showed signals related with the presence of protons and carbons in gamma-PGA molecular structure. These analyses identified several chemicals markers that indicate the polymer extract as gamma-PGA.

Keywords: *Bacillus natto;* chemical characterization; FTIR spectroscopy; gamma-PGA; NMR spectroscopy

FULL PAPER ACCEPTED by HAYATI JOURNAL

Effect of furnish materials on temperature and vapor pressure behavior in the center of mat panels during hot-pressing

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ABSTRACT - The particleboard achieves its overall performance during hot pressing process. As this process is influenced by several factors particularly the temperature and pressure, the behavior of both of them are very important to be understood. This study investigates the effects of furnish materials on the temperature and vapor pressure behavior inside the mat particleboard panels during hot-pressing. Strand type particle from hinoki and hammer-milled particle of recycled wood were used as furnish for laboratory-scale particleboard panels with the target density of 0.76 gcm-³. Mat panels with moisture content of about 10% were hot-pressed at platen temperature of 180 °C and initial pressure of 3 MPa up to the mat center reaches the same temperature of the platen temperature. PressMAN Lite (Alberta Research Council) device was used for detecting the temperature and vapor pressure change in the center of mat panels. The study shows that the furnish type affected the temperature and vapor behavior inside the mat panels. Particleboard made of hinoki strand results in longer plateau time, higher plateau temperature and higher gas pressure generated during hot-pressing than that of hammer-milled recycled wood particle. The mixed-board resulted the value between those two different furnish materials.

Keywords: furnish type; mat panels; hot-pressing; temperature; vapor pressure

FULL PAPER ACCEPTED by ITB JOURNAL

Conversion of wood fiber to High Refined Cellulose using nitric acid, sodium hydroxide and hydrogen peroxide as the delignificating agent

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ABSTRACT - High Refined Cellulose (HRC) is a cellulosic materials with a cellulose content higher than 90%. HRC may be further converted to cellulose-acetate, cellulose-nitrate, Carboxy-Methyl-Cellulose (CMC) or other cellulose base chemicals. As a renewable material, sago wood fiber waste, sugar cane fiber waste (baggase) and palm oil fiber waste have a huge potential as raw material for production of HRC and cellulose chemicals derivatives such as CMC-emulsifier, nitrocellulose-biomedical, cellulose-acetate addesive and nitrocellulose single base munition. In this study, I optimized the conditions of the chemical conversion of the wood fiber to HRC using the nitric acid, sodium hydroxide and hydrogen peroxide as the delignificating agent. The chemical processes were carried out in a 1000 mL reactor capacity, equipped with stirrer and temperature controller. Three process steps were involved in the delignification process of wood fiber; firstly using the nitric acid solution, secondly using the sodium hydroxide solution and finally using the hydrogen peroxide solution. The result shows that the delignification process of wood fiber producing HRC with cellulose content of higher than 94% were succesfully done in a 3 steps of wood fiber delignification process. The 1st step was using the HNO_3 solution, the 2nd step was using the NaOH solution and the 3rd step was using the H_2O_2 solution as the delignificating agents. The optimal process condition of this wood fiber conversion to HRC with cellulose content of higher than 94 % was achieved in processes with combinations of the HNO_3 concentration of higher than 4,5 % with ratio of the HNO_3 solution to wood fiber of higher than 25 mL/g in the 1st step, and the NaOH concentration of 1.8 N with ratio of the NaOH solution to wood fiber of 15 to 20 mL/g in the 2nd step, and the H_2O_2 concentration of higher than 3,5 % with ratio of the H_2O_2 solution to wood fiber of higher than 25 mL/g in the 3rd step.

Keywords*:* high refined cellulose, hydrogen peroxyde, nitric acid, sodium hydroxide, wood fiber conversion. Introduction

Introduction

The capture of solar energy as fixed carbon in biomass via photosynthesis, during which carbon dioxide, CO_2, were converted to organic compound, is the key initial step in the growth of biomass.

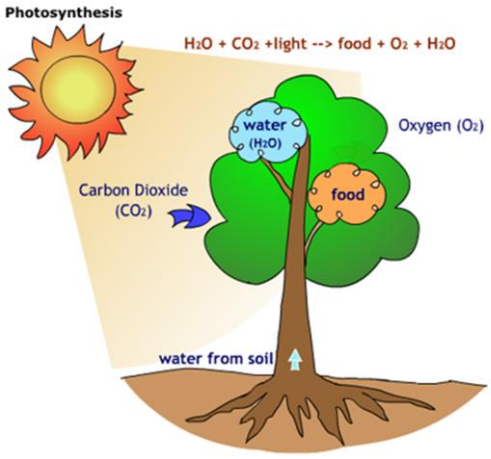

Figure 1. Conversion of carbon dioxide and water to organic component

$$n\,CO_2 + n\,H_2O \xrightarrow[solar\,energy,\,chlorophyll]{} (CHO)n + n\,O_2 \qquad (1)$$

As shown in Figure 1 and Equation (1), the Carbohydrate and cellulose in food represented by the building block $(CHO)_n$, is the organic product, so the plantation cellulose is one of the renewable chemical, a product of the photosynthesis process done by the chlorophyll in the green tree leafs. Although there are still many unanswered questions regarding the detailed molecular mechanism of photosyntesis, the prerequisites for virgin biomass growth are well established; CO_2, solar energy - light in the visible region of electromagnetic spectrum, the sensitizing catalyst chlorophyll, and living-plant are essential. Klass (1988) reported that the cupture limit of the efficiency of the incident solar radiation in biomass has been variously estimated to range from about 8% to as high 15%, but in most actual situations, it's generally in the 1% range or less. It is evident that since the simple sugars are the initial products of photosynthesis, they are the primary precursors of all the organic components in biomass. The patways to the high-molecular-weight polysaccharides involve successive condensations of the monosacharides, mainly the C-6 hexoses to yield cellulose and starches, and mainly the C-5 pentoses to yield hemicelluloses. Celluloses are composed of β-glucosidic units in the polymer chain, and starches are composed of α-glucosidic units. Glucose is the dominant immediate precursor of the celluloses.

The utilization of plant fiber as a raw material of cellulose base product or their derivatives will have a guarante that the raw material process production is formed sustainable naturally. As mention above, the cellulose of natural fiber is one of the product of photosynthesis process, which is a natural convertion of water molecule (H_2O) and Carbon dioxide (CO_2) by chlorophyll in the plantation leafs involving Solar energy. This cellulose fiber generally is the most dominant organic components in most biomass. In some parts of platation, i.e. the sugar cane stems, the palm oil branch and fruit, and in the sago stems, the cellulose content reached to as high as 50% in dry basis. In the production of sago powder, there will be produced sago fiber as solid waste. In addition to baggase from sugar cane industries, empty palm oil branch and palm oil fiber waste from palm oil industries, the sago fiber waste place its self as the potential source of cellulose in the HRC production from fiber wastes. BPS

(2011) reported that in 2011, Indonesia with the production of sugar cane as much as 2 million ton, there would be produced solid-waste-baggase as much as 10 million ton. Similar to Indonesia palm oil industries, in the production capacity of 14 million ton, at least would formed as much as 20 million ton of palm oil empty branch which were disposed as solid waste by the palm oil industries. Sago plantation has growing fast in Indonesia, especially in West Irian or Papua island. About 50 % of the world production of Sago powder comes from this region.

The solid waste from sugar cane, palm oil and sago industries may be accounted as a potential raw materials for HRC production, which can be converted further to some of end product, Cellulose Acetate, Nitro Cellulose, Carboxy Methyl Cellulose, viscous cellulose and others. Generally, the chemical process involves in HRC production is called delignification, in which the chemical reagent would destruct the lignin in fiber material and leaving the relatively pure cellulose in solid phase as HRC product. The conversion of these solid waste materials to HRC and its derivetives would certainly improve the contribution of the agroindustries to the world. Supranto (2011) reported that sago fiber can be converted to nitrocellulose through delignification and nitration processes. Supranto (2012) has studied the use of HNO3, NaOH and H_2O_2 as delignificating agent to remove the lignin in natural fiber, leaving the cellulose in solid phase as the end product of delignification process.

Materials and methods

To determine the optimal condition of the chemical processes of wood fiber waste conversion to HRC, the laboratory experimental work need to carry out. The parameters which were investigated are the concentration of the delignification agent of HNO_3, NaOH and H_2O_2, and the ratio of the amount of delignification agent (HNO_3, NaOH and H_2O_2) to the amount of the wood fiber raw material. The Research flow diagram of the fiber conversion to HRC is shown in Figure 2.

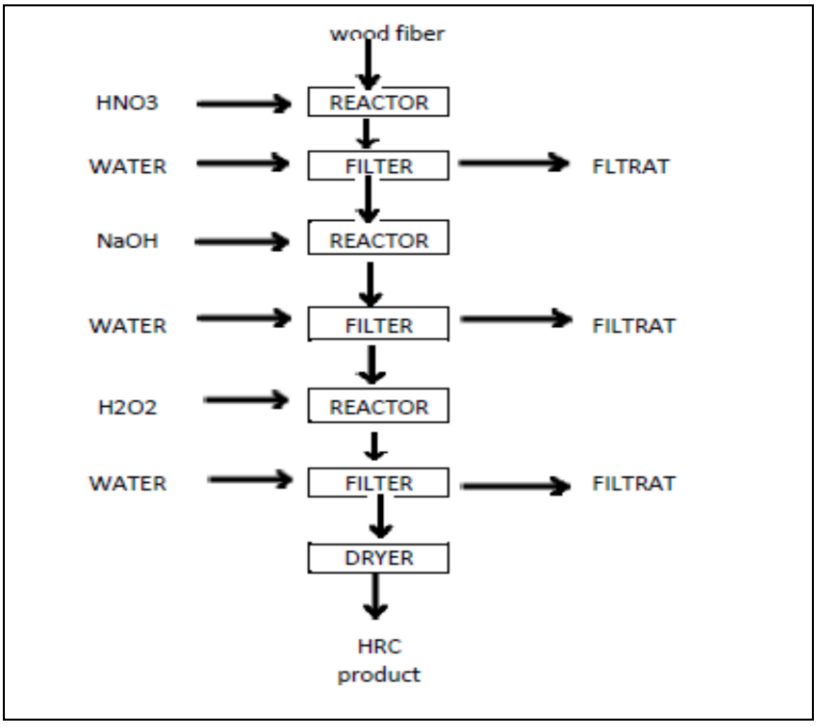

Figure 2. Flow Diagram of the experimental wood fiber delignification

Methods

The experimental work consisted of 3 process steps. In the 1st step the HNO_3 solution were used with concentration of between 2.5% to 10%, while the amount ratio of the HNO_3 solution to wood fiber were varied between 15 to 45 mL/g. In the 2nd step the NaOH solution were used with NaOH concentration in the range of 1 N to 2.5 N and the amount ratio of NaOH solution to wood fiber were varied between 15 to 45 mL/g. In the 3rd step the H_2O_2 solution with the concentration varied from 1% to 4%, with the amount ratio of H_2O_2 to wood fiber in the range of 15 to 45 mL/g. When the 3rd step of delignification process condition was optimized, the process conditions of the 1st and 2nd process step were kept fixed. The experimental work consist of 48 runs, the first 16 run generated data which will indicate the effect of H_2O_2 parameter in the delignification process step-3, the second 16 run generated data indicating the effect of NaOH parameter and the last 16 run generated data the effect of HNO_3 parameter on the cellulose content in the HRC product.

Data analysis

The experimental result data which were the concentration of cellulose in HRC product, were interpolated by mean of second order polynomial correlation, producing a set of interpolated matrix data. Then the matrix data were analyzed and plotted as 3D graphically picture. The optimization graphically method were used to generate 2D contour plots of cellulose content in HRC versus 2 simultaneous parameter investigated, which are concentration of the delignification agent (HNO_3, NaOH and H_2O_2) and the volume ratio of the delignification agent to the weight of the wood fiber.

Results and discussion

The effect of H_2O_2 to wood fiber ratio and H_2O_2 concentration on the delignification process step-3.

Table 1 is the experimental result of the effect of H_2O_2 concentration (C_3) and ratio of H_2O_2 to wood fiber (R_3) on the delignification process step-3, with process temperature of 80 ^oC, wood fiber of 10 g, process duration time of 2 hour. When the 3rd step of delignification process was optimized, the operating condition of the 2nd and the 1st step were kept fixed. The data in Table 1 were analysed and the result were represented in Figure 3. The correlation formulas between the cellulose content in HRC (P) and the H_2O_2 concentration (x) for ratio H_2O_2 to wood fiber (R_3) value of 15, 25, 35, and 45 are represented by the equations 2 to 5 respectivelly.

$$P = -1.6659\,x^2 + 13.275\,x + 65.466 \quad (2)$$

$$P = -3.1050\,x^2 + 23.369\,x + 49.554 \quad (3)$$

$$P = -3.8150\,x^2 + 28.182\,x + 40.703 \quad (4)$$

$$P = -2.9490\,x^2 + 22.611\,x + 48.379 \quad (5)$$

Tabel 1. The H_2O_2 effect on HRC product, with wood fiber fixed of 10 gram, duration time of 2 hour and temperature of 80 ^oC.

No.	HNO_3		NaOH		H_2O_2		HRC
	C_1, (%)	R_1 (mL/g)	C_2, (N)	R_2 (mL/g)	C_3, (%)	R_3 (mL/g)	Cellulose(%)
1	7.5	25	2	25	3.0	15	90.42
2	7.5	25	2	25	3.5	15	91.16
3	7.5	25	2	25	4.0	15	92.28
4	7.5	25	2	25	4.5	15	91.35
5	7.5	25	2	25	3.0	25	91.84
6	7.5	25	2	25	3.5	25	91.93
7	7.5	25	2	25	4.0	25	93.73
8	7.5	25	2	25	4.5	25	91.71
9	7.5	25	2	25	3.0	35	91.11
10	7.5	25	2	25	3.5	35	92.02
11	7.5	25	2	25	4.0	35	92.98
12	7.5	25	2	25	4.5	35	90.07
13	7.5	25	2	25	3.0	45	89.67
14	7.5	25	2	25	3.5	45	91.39
15	7.5	25	2	25	4.0	45	91.64
16	7.5	25	2	25	4.5	45	90.41

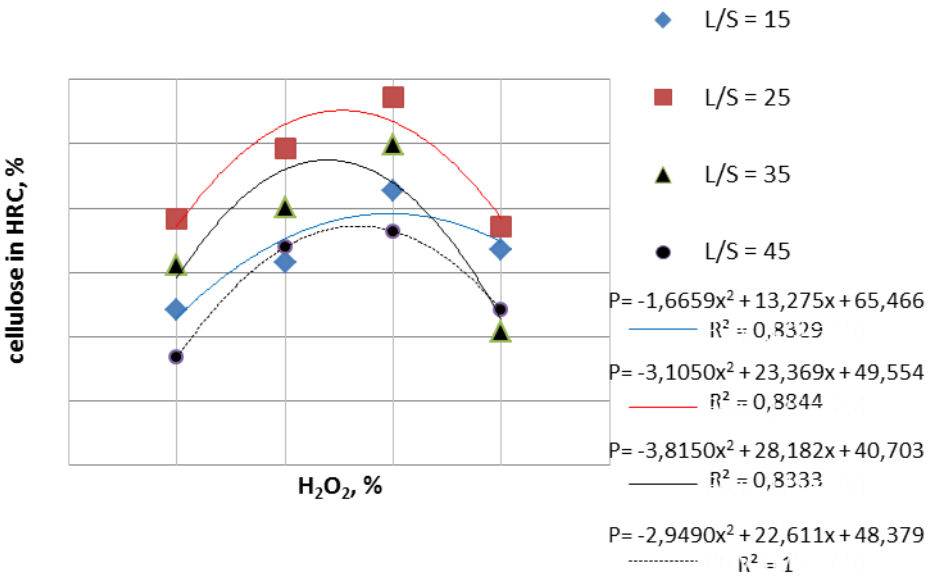

Figure 3. The effect of H_2O_2 to wood fiber ratio and H_2O_2 concentration on the cellulose content in the HRC product

Using the equations 2 to 5 as the representatif equations of the the corellation formulas which corellate the H_2O_2 concentration and the cellulose content in HRC with variation of the ratio volume of H_2O_2 solution to the weight of Sago fiber, then the 3D graphically picture of the effect of H_2O_2 in delignification process can be performed (Figure 4 and 5). Figure 4 and 5 showed the cellulose content in HRC product is increasing with the increase of the H_2O_2 concentration from 3.0% to 4.0%, then this value is slighly decreasing when the H_2O_2 concentration higher than 4.0% was used. As shown in Figure 4, the optimal process condition to produce HRC with cellulose content of higher than 93% can be achieved by using a combination of H_2O_2 concentration of higher than 3.5% and H_2O_2 to Sago wood fiber ratio of higher than 20 mL/g. The H_2O_2 concentration of 4% and H_2O_2 to

Sago wood fiber ratio of 25 mL/g , then, were used in the next experimental optimization of the delignification process step-2 and process step-1.

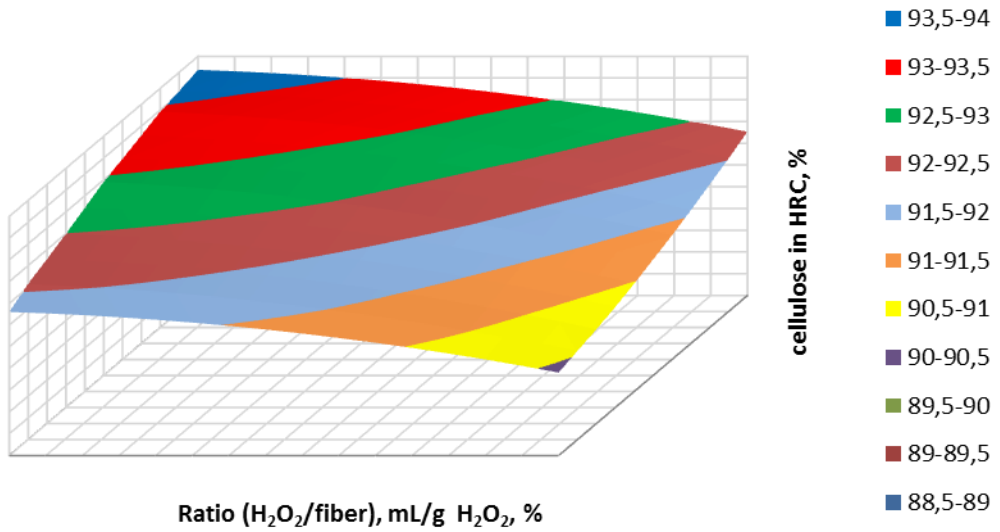

Figure 4. The effect of H_2O_2 to wood fiber ratio and H_2O_2 concentration on the cellulose content of HRC product

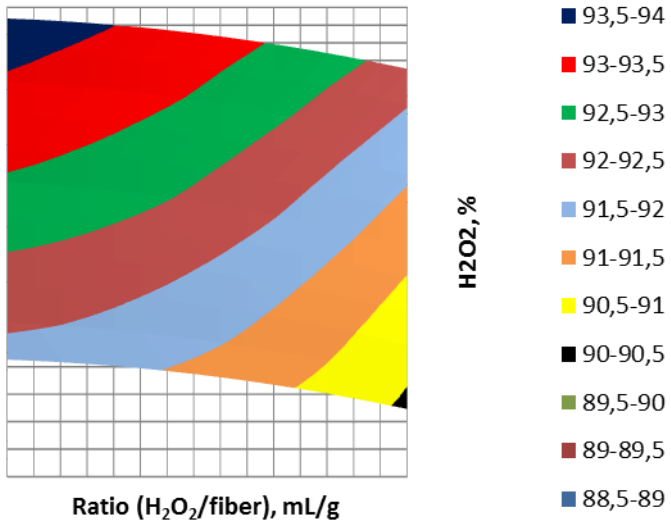

Figure 5. The simultaneous effect of the H_2O_2 to wood fiber ratio and H_2O_2 concentration on the cellulose content in HRC product

The effect of NaOH to wood fiber ratio and NaOH concentration on the delignification process step-2.

Table 2 is the experimental result of the effect of the NaOH solution to wood fiber ratio (R_2) and the NaOH concentration (C_2) on the delignification process step-2, using process temperature of 80^{o}C, solid wood fiber of 10 g, duration time of 2 hr, following by the 3rd process step involving H_2O_2 with the optimal process conditions determined previously. The data shown in Table 2 were analysed and represented in Figure 6 and 7. The corellation formulas between the NaOH concentration (y) with the cellulose content in HRC (P) in various ratio of NaOH to wood fiber (R_2) value of 15, 25, 35 and 45

$$P = -2.410\,y^2 + 8.5970\,y + 85.987 \quad (6)$$

$$P = -1.975\,y^2 + 6.5835\,y + 87.083 \quad (7)$$

$$P = -2.345\,y^2 + 8.4085\,y + 84.368 \quad (8)$$

$$P = -1.750\,y^2 + 6.7190\,y + 84.293 \quad (9)$$

Tabel 2. The effect of NaOH on HRC product, with wood fiber fixed of 10 gram, duration time of 2 h and temperature of 80 ^{o}C.

No.	HNO_3		NaOH		H_2O_2		HRC
	C_1, (%)	R_1 (mL/g)	C_2, (N)	R_2 (mL/g)	C_3, (%)	R_3 (mL/g)	Cellulose(%)
17	7.5	25	1.0	15	Opt	Opt	92.07
18	7.5	25	1.5	15	Opt	Opt	93.77
19	7.5	25	2.0	15	Opt	Opt	93.23
20	7.5	25	2.5	15	Opt	Opt	92.52
21	7.5	25	1.0	25	Opt	Opt	91.84
22	7.5	25	1.5	25	Opt	Opt	92.70
23	7.5	25	2.0	25	Opt	Opt	92.80
24	7.5	25	2.5	25	Opt	Opt	91.05
25	7.5	25	1.0	35	Opt	Opt	90.65
26	7.5	25	1.5	35	Opt	Opt	91.05
27	7.5	25	2.0	35	Opt	Opt	92.46
28	7.5	25	2.5	35	Opt	Opt	90.52
29	7.5	25	1.0	45	Opt	Opt	89.19
30	7.5	25	1.5	45	Opt	Opt	90.65
31	7.5	25	2.0	45	Opt	Opt	90.51
32	7.5	25	2.5	45	Opt	Opt	90.23

It can be seen in Figure 6 and Figure 7 show that the cellulose content in HRC product is increasing with the increase of NaOH concentration from 1N to 2N, then this value is slightly decreasing when NaOH concentration higher than 2N. Figure 7 also shows the optimal process condition to produce HRC with cellulose content higher than 93% could be achieved by using a combination of 2N NaOH (C_2) and NaOH to wood fiber ratio (R_2) of higher than 20 mL/g. 2N NaOH and NaOH to wood fiber ratio of 25 mL/g were used in the next experimental optimization of the delignification process of step-1.

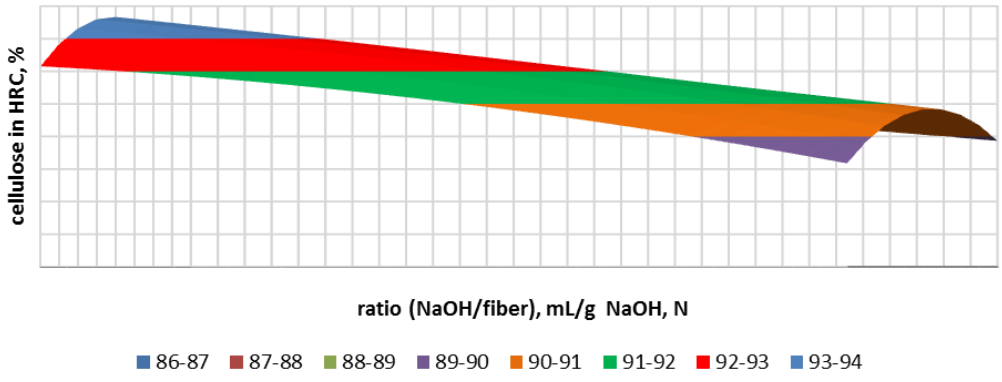

Figure 6. The effect of NaOH to wood fiber ratio and NaOH concentration on the cellulose content in the HRC product

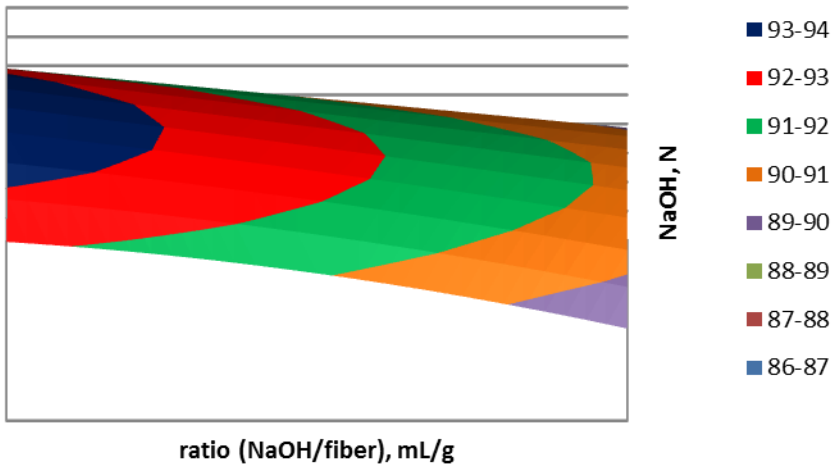

Figure 7. The simultaneous effect of NaOH to wood fiber ratio and NaOH concentration on the cellulose content in HRC product

The effect of HNO_3 to wood fiber ratio and HNO_3 concentration on delignification process step – 1.

Table 3 is the experimental result of HNO_3 effect on Sago wood fiber ratio and HNO_3 concentration on the delignification process step-1, using temperature of 80 ^oC, Sago wood fiber of 10 g, duration time of 2 h, following by second step delignification involving NaOH in step-2 and H_2O_2 in step-3 with the optimal processes conditions previously determined. The data shown in Table 3 were analysed and represented in Figure 8 and 9. The corellation formulas between HNO_3 concentration (z) with the cellulose content in HRC (P) in various ratio of HNO_3 to wood fiber (R_3) value of 15, 25, 35 and 45 mL/g are represented by the equation 10 to 13 respectivelly.

$$P = -0.0093\,z^2 + 0.2047\,z + 93.280 \quad (10)$$

$$P = -0.0107\,z^2 + 0.2233\,z + 93.064 \quad (11)$$

$$P = -0.0078\,z^2 + 0.2013\,z + 92.874 \quad (12)$$

$$P = -0.0125\,z^2 + 0.2475\,z + 91.695 \quad (13)$$

Tabel 3. The effect of HNO_3 on HRC product, with wood fiber fixed of 10 gram, duration time of 2 h and temperature of 80 ^oC.

No.	HNO_3		NaOH		H_2O_2		HRC Cellulose (%)
	C_1, (%)	R_1 (mL/g)	C_2, (N)	R_2 (mL/g)	C_3, (%)	R_3 (mL/g)	
33	2.5	15	Opt	Opt	Opt	Opt	92.22
34	5.0	15	Opt	Opt	Opt	Opt	92.66
35	7.5	15	Opt	Opt	Opt	Opt	92.81
36	10.0	15	Opt	Opt	Opt	Opt	92.93
37	2.5	25	Opt	Opt	Opt	Opt	93.20
38	5.0	25	Opt	Opt	Opt	Opt	92.72
39	7.,5	25	Opt	Opt	Opt	Opt	92.73
40	10.0	25	Opt	Opt	Opt	Opt	94.06
41	2.5	35	Opt	Opt	Opt	Opt	93.71
42	5.0	35	Opt	Opt	Opt	Opt	93.15
43	7.5	35	Opt	Opt	Opt	Opt	94.22
44	10.0	35	Opt	Opt	Opt	Opt	94.43
45	2.5	45	Opt	Opt	Opt	Opt	93.53
46	5.0	45	Opt	Opt	Opt	Opt	93.98
47	7.5	45	Opt	Opt	Opt	Opt	94.08
48	10.0	45	Opt	Opt	Opt	Opt	94.25

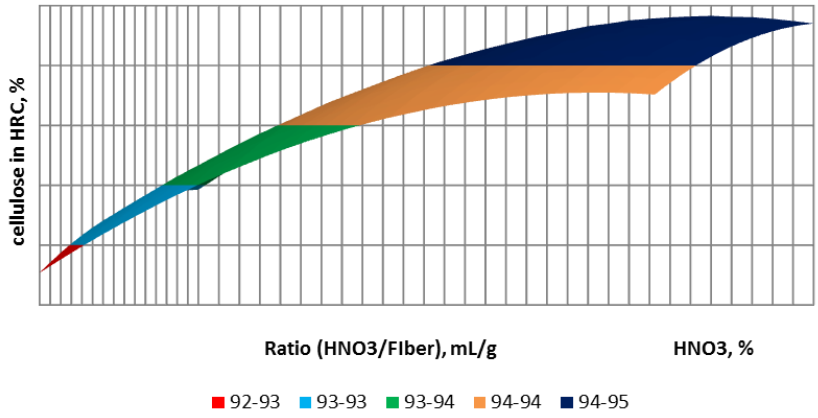

Figure 8. The effect of HNO_3 to wood fiber ratio and HNO_3 concentration on cellulose content in HRC product

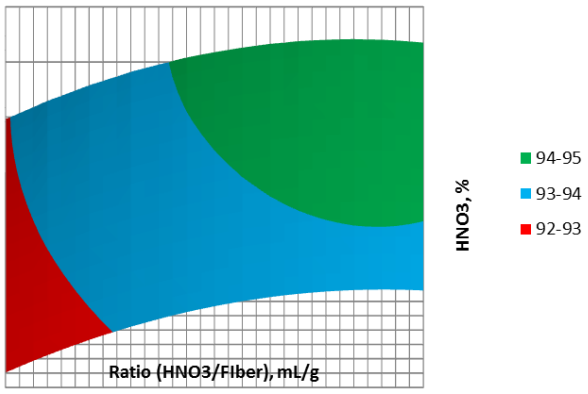

Figure 9. The simultaneous effect of HNO_3 to wood fiber ratio and HNO_3 concentration on cellulose content in HRC product

Conclusion

Conversion of wood fiber to HRC with cellulose content of higher than 94% were succesfully achieved in a 3 steps of wood fiber delignification process. The 1st and 2nd steps used HNO_3 solution, while 3rd step used H_2O_2 solution as the delignificating agents.

The optimal process condition of this wood fiber conversion to HRC with cellulose content of higher than 94% was achieved in processes with combinations of HNO_3 concentration higher than 4,5% with the ratio of HNO_3 solution to wood fiber of higher than 25 mL/g in the 1st step, and NaOH concentration of 1.8 N with the ratio of NaOH solution to wood fiber of 15 to 20 mL/g in the 2nd step, and the H_2O_2 concentration of higher than 3,5% with the ratio of the H_2O_2 solution to wood fiber of higher than 25 mL/g in the 3rd step.

Nomenclature (if necessary)

List the nomenclature in alphabetical order. List Roman letters followed by Greek symbols followed by subscript and superscripts.

C_1	=	Consentration of HNO_3 in process step-1
C_2	=	Concentration of NaOH in process step-2
C_3	=	Concentration of H_2O_2 in process step-3
HNO_3	=	Nitric Acid
HRC	=	High Refined Cellulose
H_2O_2	=	Hydrogen Peroxyde
NaOH	=	Sodium Hydroxide
P	=	Cellulose content in HRC product
R_1	=	Ratio amount of the HNO_3 solution to the wood fiber, mL/g
R_2	=	Ratio amount of the NaOH solution to the wood fiber, mL/g
R_3	=	Ratio amount of the H_2O_2 solution to the wood fiber, mL/g
x	=	H3O2 concentration, g/L
y	=	NaOH concentration, g/L
z	=	HNO_3 concentration, g/L

Reference

BPS, 2011

Klass D.L., *Biomass for Renewable Energy*, Academic Press Limited, California, USA, 1988.

Supranto, *Converting of the Sago Fiber to Nitrocellulose by delignification and nitration processes*, AUNSEED-Net Manila, Philipine Conference, 20-21 *January* 2011.

Supranto, *Pembuatan Selulose Kemurnian Tinggi dari Serat Alami Tanaman dengan Menggunakan HNO_3, NaOH dan H_2O_2 sebagai Agen Proses Delignifikasi*, Proceeding, Seminar Nasional Teknik Kimia Indonesia dan Musyawarah Nasional APTEKINDO 2012, The chalenge of Chemical Engineering Institutions in Product Innovetion for a Sustainable Future, Universitas Indonesia, Jakarta Indonesia, 2012.

FIBER - 04

Decay Resistance of Medium Density Fiberboard (MDF) Made From Pineapple Leaf Fiber

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ABSTRACT - Medium Density Fiberboard (MDF) production is increasing due to the development of manufacturing technologies. MDF products are utilized in traditional wood applications that require fungal resistance. This study investigated some of the important biodegradation properties of MDF composite made from reneable biomass of pineapple leaf fiber (*Ananas comosus*). Variable factors were type of board and type of resin. Two diffrenet types of board with a target density of 0.8 gr/cm^3 were manufactured. The board was prepared in three layers of about 1:1:1 weight ratio in cross-oriented and unidirectional board using low molecular weight (LM) Phenol Formaldehyde (PF) resin type PL-3725 and high molecular weight (HM) PF resin type PL-2818 for impregnation and adhesive purposes. Decay resistance (white and brown rot fungi) of the MDF were evaluated in order to assess its biological performance. In this study, fiber orientation had no effect on both decay resistance of white and brown rot fungi. However, a slight increase was found for the mass loss of the high molecular weight PF compared with mix low and high molecular PF resin. The total resin content of 20% of the type a boards prohibits the degradation by decay.

Keywords: decay resistance; medium density fiberboard (MDF); pineapple leaf fiber; phenol resin (PF)

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FIBER - 05

Synthesis and Characterization of Bio-based Nanomaterial from Jabon (*Anthocephalus cadamba* (Roxb.) Miq) Wood Bark : Organic Material Waste from Community Forest

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ABSTRACT - The application of nanotechnology to produce nanomaterial from renewable bio-based material like wood bark, has a great potential for forest products industry. To support this issue, we investigate the production of bio-based nanomaterial using conventional balls milling. Jabon (*Anthocephalus cadamba* (Roxb.) Miq) wood bark, organic material waste from community forest was subjected to conventional balls milling for 96 h and was converted into bio-based nanomaterial. The morphology and particles size, chemical components, functional groups and crystallinity of bio-based nanomaterial were evaluated using scanning electron microscopy (SEM), scanning electron microscopy that extended with energy dispersive X-ray spectroscopy (SEM-EDS), Fourier Transform Infrared Spectroscopy (FTIR) and X-ray Diffraction (XRD). The particles size was obtained between 43 nm up to 469 nm and in the range of 10-1000 nm. The chemical components found in bio-based nanomaterial from JWB were carbon, oxygen, chloride, potassium and calcium.

Keywords: bio-based nanomaterial, conventional balls milling, jabon wood bark.

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FIBER - 06

Seedling Quality and Early Growth of *Paraserianthes falcataria* (L) Nielsen F-2 Half-Sib Plant in Progeny Test

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ABSTRACT - The research was conducted to find the effects of parental and site to seedling growth of *Paraserianthes falcataria* (L) Nielsen F-2 half-sib in progeny test at Jatinangor Sumedang, West Java. The seedlings of *P. falcataria* have been planted with spacing 1m x 3m. The experimental design which used in this research was Randomized Complete Block Design with row plot system. There were two factors which studied, 16 of parentals and 4 blocks. Seedling growth variables measured were height, diameter, number of leaf, sturdiness and adaptability of seedlings in the field. This study showed that quality of *P. falcataria* seedling could not be classified based on seedling quality standard, but its growth quite well. Height, diameter and number of leaf were influenced by parental, site and their interaction. Adaptability of progeny of mother tree-1 from Subang provenance (parental-5 or SuP1B1), mother tree-1 from Kediri provenance (parental-10 or KeP1B2) and mother tree-2 from Kuningan provenance (parental-13 or KuP2B2) were lowest, whereas progeny of mother tree-1 from Kediri provenance (parental-4 or KeP1B1) had highest adaptability.

Keywords: early growth, *Paraserianthes falcataria*, parental, progeny test, seedling quality.

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FIBER - 07

Preliminary Evaluation On Genetic Variation of Two Year Old Surian (*Toona Sinensis* Roem) Progeny Test Plants Assessed by RAPD Marker

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ABSTRACT - Study on genetic variation of surian (*Toona sinensis* Roem) plants is still limited. The genetic information is needed for the tree improvement program. Genetic variation of tree population can be estimated through morphological or molecular markers. Estimation using morphological markers is time consuming, whereas by molecular marker, the estimation of genetic variation can be carried out faster. One of the molecular markers commonly used as a first step to estimate tree genetic variation is the so-called RAPD (Random Amplified Polymorphic DNA). This research was done aiming at determining genetic variation of 2 year old surian offsprings in the progeny test using RAPD marker. Surian leaves were used as material for RAPD analysis which were sampled from 18 individuals. Three out of 6 primers tested for RAPD analysis produced clear and reproducible bands, namely OPY-19, OPY-13, and OPO-10. Polymorphic bands resulted from the RAPD profiles were then analyzed using POGENE and NTSYSpc softwares. Results showed that genetic variation (heterozygosity, h_e) and Shanon index (i) values for Surian population were 0.2446 and 0.3728, respectively. The number of polymorphic loci (NPL) was 10, percentage of polymorphic loci (PPL) was 62.5%, number of alleles observed (na) was 1.7500 and number of alleles effective (ne) was 1.4007. Genetic distances among accessions varied from 0.000 (between seedlots from Sumedang_4, Pekalongan, and Sumedang) to 0.6932 (between families from Wonosobo and Kuningan_4). Individual clustering based on genetic distance using UPGMA method showed two major clusters at 30% genetic diversity level. Seedlot from Central Java were clustered to one cluster, while seedlot from West Java were clustered into two clusters. This study indicated that the seedlot from West Java is more diverse than the one from Central Java. Further investigation using other marker types, i.e. AFLP, will be carried out to confirm these prelimnary findings.

Keywords: Genetic variation, Toona sinensis, genetic variation, RAPD, heterozygosity.

FULL PAPER WITHDRAWED by AUTHOR

Effect of Board Type on Some Properties of Bamboo Strandboard

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ABSTRACT - The objective of this study was to evaluate the properties of bamboo strandboard (OSB) at different board types and strand-lengths. Two types of strand-length and MDI resin were used to produce three types of strandboard. The bending properties and dimensional stability of bamboo strandboard were evaluated according to Japanese Industrial Standard (JIS) for particleboard, and the results were summarized as follows. The bending properties and internal bond strength were affected by board types and strand-length. Uneven resin distribution on 80 mm strandboard compared with 50 mm strandboard will affect the value of internal bond. For better value of internal bond especially for longer strand, glue bending technology is needed to be improved. Thickness swelling (TS) of RAND-board was the highest compared to other boards, and linear stability is affected substantially by strand alignment. The RAND-board and cross-oriented at core of 3LAY-board effectively restrained the LE in direction of perpendicular strand alignment. The cross-oriented core may be the most effective way to reduce this dimensional change and decrease the bending property value in perpendicular directions

Keywords: bamboo, strandboard, long strand, board type, bending properties, dimensional stability.

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FOOD - 01

Embryo Incision as a New Technique to Double Seedling Production of Indonesian Elite Coconut Type"Kopyor"

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ABSTRACT - One of the major limitation of seedling production of kopyor-type coconut using embryo culture technique is that only one seedling can be produced from a single embryo. Therefore, we report on the development of a new break trough technique to produce double seedling from a single embryo. The technique was achieved through four steps, viz. (i) germination; (ii) incision; (iii) splitting and (iv) recovering. The histological work has also carried out on the development of halved embryo into a new shoot. The best recovery process were obtained when the incised embryos were splitted become two and recovered into Murashige and Skoog (MS) medium supplemented with 2 µM IBA and 15 µM kinetin. Following this protocol, the average of 56 shoots was successfully recovered out of 30 zygotic embryos. Histological study also revealed that the meristem tissue of the halved embryo could produce new meristem and primordial leaf. Most of the shoots then underwent to produce normal seedlings and can be acclimatized successfully after having 2 or 3 leaves. This protocol is useful for the routine seedling production of kopyor-type coconut.

Keywords: Embryo culture, embryo splitting, multiplication, in vitro culture, meristem

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Induction of Somatic Embryos From Leaf and Stem Nodal Section of Potato (*Solanum tuberosum* L.)

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ABSTRACT Potato is one of the most important horticultural commodities in Indonesia. Recently, market demand for potato comodities increase in line with the increase of population of Indonesia. However, the availability of qualified potato seedling are still not able to meet market demand. To solve these problems, tissue culture through somatic embryogenesis, can be used as one of alternative method to obtain high quality of potato seedling problem. It is therefore, this research has been done with objectives to evaluate the most potential type of explants to produce potato somatic embryos, to evaluate the combinations of growth regulator (2,4-D and BAP) at various concentration for potato somatic embryos induction, and to evaluate the ability of BAP for somatic embryos maturation process. Young leaf and stem nodal section were used as a source of explants. These explants were cultured in MS media suplemented with 9 µM 2,4-D and 4.5 µM BAP for leaf explants, or 9 µM 2,4-D and 1 µM BAP for stem nodal section. Developing calli were then transferred to liquid MS media supplemented with several concentration (1 - 10) µM of 2,4-D and BAP (0.5 – 10) µM for induction of somatic embryos. For embryos maturation process, embryogenic callus/somatic embryos from induction medium were transferred to embryos maturation medium, which consisted of MS medium supplemented with (1 – 5) µM BAP. The results showed that the best media for inducing potato somatic embryos derived from leaf explant was MS with growth regulator composition (2,4-D 1 µM + BAP 10 µM); (2,4-D 1 µM + BAP 5 µM);or (2,4-D 2,5 µM + BAP 5 µM), meanwhile for inducing somatic embryos derived from stem nodal section explant was MS media supplemented with (2,4-D 1 µM + BAP 0.5 µM);(2,4-D 5 µM + BAP 1 µM);or (2,4-D 10 µM + BAP 5 µM). In the maturation process, somatic embryos derived from leaf as well as stem nodal section explant were well-developed in MS media supplemented with 5 µM BAP. Two types of explants were tested, explants stem nodal section was able to produce the highest percentage of globular embryos (16.3%) compared with leaf explants (10.6%). In the maturation stage, the highest percentage of embryos heart (10.3%) and torpedo (4%) obtained from explants stem nodal section. Based on the results, it could be concluded that the most potential type of explant for generating potato somatic embryos was stem nodal section. The highest number of somatic embryos was induced from stem nodal section in the media containing 2,4-D 1µM + BAP 10 µM, and the highest development/maturation rate of somatic embryos was obtained from stem nodal section explants in media containing 5 µM BAP.

Keywords: growth regulator, potatoes (*Solanum tuberosum* L.), somatic embryogenesis, tissue culture

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FOOD - 03

Cloning of *P5CS* Gene from *Saccharum officinarum* L.

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ABSTRACT - Abiotic stresses, such as drought, cause negative impacts on crop adaptation and productivity. On the other hand, sugarcane productivity can be increased by extensification method which include the use of marginal land. To boost the sugar production and extend its range, we seek to improve its stress tolerance by genetic engineering method. Under abiotic stress, proline, an osmoprotectant compound, will be accumulated to stabilize cell membrane and prevent protein degradation. P5CS ($^{\Delta}$1-pyrroline-5-carboxylate synthetase) is one of the key regulatory enzyme in proline biosynthesis. We isolated and registered the *P5CS* gene from *Saccharum officinarum* L (Genbank Accession Number : KF178300). The gene was isolated by reverse transcriptase PCR using spesific primers. The primers were designed based on nucleotide sequence of *P5CS* gene from *Saccharum officinarum* (Genbank Accession Number : EF155655). The gene has 98% homology with *P5CS* gene from *S. arundinaceum* (*SaP5CS*, Genbank Accession Number : EU113257), 97% homology with *P5CS* gene from *Saccharum officinarum* (*SoP5CS*, Genbank Accession Number : EF155655), and 93% homology with P5CS2 gene from Sorghum bicolor (*SbP5CS2*, Genbank Accession Number : GQ377720). The gene encodes 729 amino acid. Multiple sequence alignment result showed that there were conserved domain glutamat-5-kinase and gamma-glutamyl phosphate reductase in amino acid sequence of the isolated sugarcane *P5CS*. Further bioinformatic analysis by filogenetic tree suggested that the isolated *P5CS* gene was similiar to *SoP5CS, SaP5CS*, and *SbP5CS2* . In order to study the function of the gene, we constructed the isolated *P5CS* gene into plant expression vector, pCAMBIA 1303, using cut and paste method. The construct in now available for further research on transgenic sugarcane. In conclusion, we succesfully isolated the full-length of *P5CS* coding sequence from sugarcane that might be potensial to be used in improving stress tolerance of sugarcane.

Keywords: isolation, sugarcane, P5CS1,abiotic stress, proline

FULL PAPER ACCEPTED by HAYATI JOURNAL

Planning of Seaweed Cultivation, *Eucheuma cottonii* to Support Sustained Development of Sentra Minapolitan in District Serang, Banten Province

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ABSTRACT. One of the commodities in Banten Province is the *Eucheuma cottonii* seaweed. This seaweed has a great chance in the local and global markets. Data obtained from the Department of Marine and Fisheries of Banten Province shows that, land use of seaweed cultivation in the Serang Minapolitan has only reached 53%, with an increase in the trend of seaweed production from 2006 to 2011 but then declined by 6.2% in 2012. Reflecting upon this trend, this study aims to formulate a development planning of the *E. cottonii* cultivation in Serang Minapolitan. The analysis is based on assessment of environmental, technical, social, and economic aspects. This was then further analysed qualitatively through a SWOT analysis and filter theory. The results showed that seaweed cultivation in Serang is indeed feasible, based on the following factors: a). The environmental conditions of cultivation area goes in accordance with the standard criteria of SNI 7579.2:2010 (environmentally); b). The longline cultivation techniques used are in accordance with the conditions of waters, in the form of alluvial sand (technically); c). Cultivation is supported by market opportunities and a strong role of government (socially) and d). NPV for ten years (10% interest rate) is IDR 223.614.298. Time refund for 13 months with an IRR of 91% (economically). The appropriate marketing programs should be based on the map position of enterprises in Quadrant III (0.74-2.09). This programs are of the following: 1). Continuity of government assistance to farmers' groups to produce derivative products preceded certification of raw material product; 2). Determination of unit processing seaweed industry in the Minapolitan.

Keywords: *Eucheuma cottonii,* Minapolitan Seaweed, Serang Regency, SWOT, Filter Theory.

Introduction

Indonesia, with a coastline of over 87,000 kilometers, is the longest beach in the world (Delinom, 2005). This serves as a great potential to be developed as one of the economic resources for coastal communities. Accordingly, the Government of Indonesia set minapolitan or city centers fisheries in 32 provinces to raise the state's economy, through the development of coastal areas. One of the minapolitan in Java includes Serang Minapolitan. This minapolitan has a supply of a seaweed called *Eucheuma cottonii*- also known as *Kappaphycus alvarezii*. This seaweed species belongs to the class of red algae (*Rhodopyceae*) (Figure 1). They are included in the group of karaginofit, which produces kappa carrageenan, a hard and stiff material (Mustamin, 2012). Carrageenan is used by the food industry as a raw material in the form of carrageenan powder. Carrageenan powder functions as an agent stabilizer, thickener (thickening agents), gelling, and emulsifyer (Yasita and Rachmawati, 2009). The carrageenan powder is made through a process of ATC (Alkaline Treated Cottonii). Indonesia itself is an exporter of seaweed *E. cottonii*, with a potential of local cultivation spreading from the coastal waters of Nanggroe Aceh Darussalam to Papua (Denantika, 2012).

Figure 1 *Eucheuma cottonii*

Eucheuma cottonii production data in Serang Minapolitan shows an increased production from the year 2006-2011. However, in 2011-2012, the number of production decreased by 6.2%. Based on aspects of land for cultivation, land availability, and ease of access to obtain ownership rights of land, the chance for seaweed cultivation in Serang is still wide open. Administrative proceedings only need permits from the village chief, which is at no charge. The Department of Marine and Fisheries of Banten Province data (2011) shows that the use of potential land for cultivating seaweed has just reached 53.7%. Based on the calculations (Setyaningsih, 2011), seaweed cultivation done with a longline method has several advantages. This includes multiple harvests (5-6 times) a year, cultivating techniques not requiring special skills, and a relatively inexpensive cost of development. In addition, seaweed *E. cottonii* is a commodity with a high level of demand from global and local market. Indonesia also supplies the need of the global market in the form of raw seaweed. But processed seaweed is still at a low level, at around 30% of total production (Rangga, pers comm). Based on those problems, this study aims to formulate a development program of the *E. cottonii* seaweed cultivation in Serang Minapolitan, based on a feasibility study of environmental, technical, social, and economic.

Materials and Methods

Location and Time of Research

The study was conducted in minapolitan region, in the sub-District of Pontang, Tirtayasa, and Tanara (Figure 2). Point location (red star) shows the location of the study, which is in the Domas Village (Pontang) and Lontar Village (Tirtayasa).

Figure 2 Location of the study area. (a) Indonesia map (Serang District is in the red circle) & (b) Serang District map (Domas and Lontar Village are in the red star)

Method of Research

This study used qualitative methods to gather primary and secondary data. Primary data are obtained through field observations, in-depth interviews, and questionnaires by respondents who have had experience in seaweed cultivating and are related to the research topic (purposive sampling). The respondents who participated are farmers, collectors, mill buyers seaweed, the Department of Marine and Fisheries of the Serang Disctrict, as well as the Banten Province. Primary data collection was conducted by specifying a search key informants through the snowball sampling technique (determination of respondents based on the respondent's prior information). Secondary data were obtained from relevant government agencies, such as the Department of Fisheries and Marine of the Banten Province, Fishery and Marine Serang Regency, Central Regional Research and Development of Banten, and the Central of Statistics of Serang. Results identification of internal and external factors was then analyzed using SWOT (Rangkuti, 2006) and filter theory. This research workflow can be seen in Figure 3.

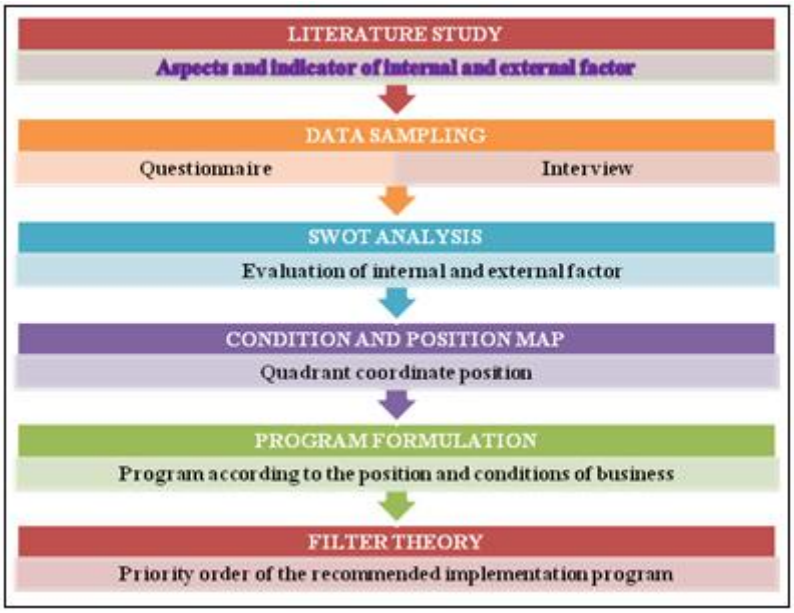


Figure 3 Flowchart stages of research

Results and Discussion

Environmental Analysis

National Standardization Agency of Indonesia has set the standard of water quality requirements for cultivating *E. cottonii*. This is based on the SNI (Indonesian National Standard) 7579.2:2010. Standardization parameters includes temperature, salinity, and pH. Table 1 presents a comparison data of the environmental conditions in the study site with the parameters of SNI 7579.2:2010. Based on these data, the condition of the waters in the cultivated area in the study site (Lontar and Domas Village) are feasible for seaweed cultivation.

Tabel 1 The average value of water quality in cultivated area compared with standard water quality requirements SNI 7579.2:2010

No.	Parameter	SNI standard of water condition for cultivating *E. cottonii*	Water conditions in Lontar Village (DKP, 2010)	Water conditions in Domas Village (Purbani *et. al.*, 2010)
1	**Temperature**	26-32 ˚C	29,8 ˚C	29.44-30.22 ˚C
2	**Salinity**	28-34 ppt	31-33 ppt	31.02-31.44 ppt
3	**pH**	7-8.5	9	7.85-8.23

Technical Analysis

The seaweed cultivation technique used in Serang Minapolitan is the longline method (Figure 4). There is a modification of the use of anchor which is instead replaced by a bamboo, to reduce investment costs. This cultivation method is in accordance with the condition of substrate, in the form of sand and sludge alluvial with water depth ranges between 1 to 5 meters.

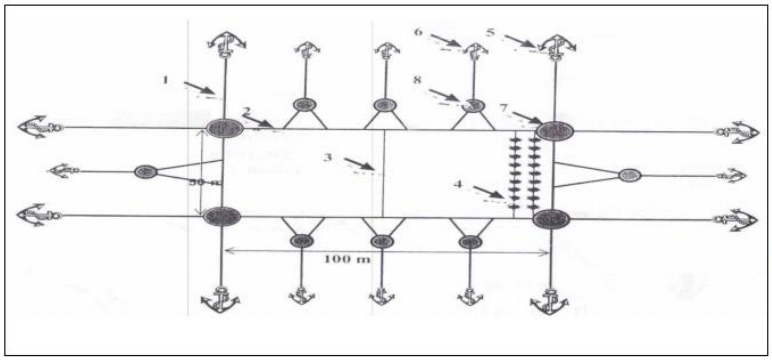


Figure 4 Long line construction framed size 100m x 50m (SNI 7579.1:2010)

Social Analysis

Seaweed farming in Serang is supported socially by a human resource potential, government support through the establishment of Minapolitan project, and high market opportunities. Market opportunities of seaweed *E. cottonii* can be seen from the projected needs of dried seaweed in the world (Figure 5). But in terms of price stability, seaweed *E. cottonii* has an unstable price, despite an upward trend (Figure 6).

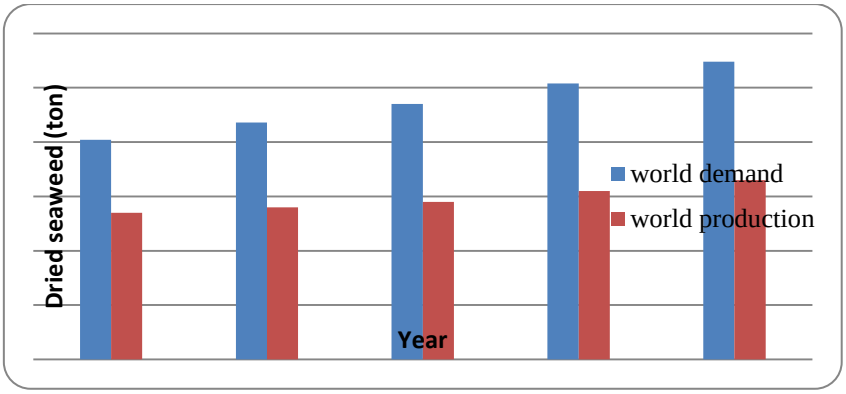


Figure 5 Projections of *E. cottonii* market opportunities (Tjitroresmi, 2009)

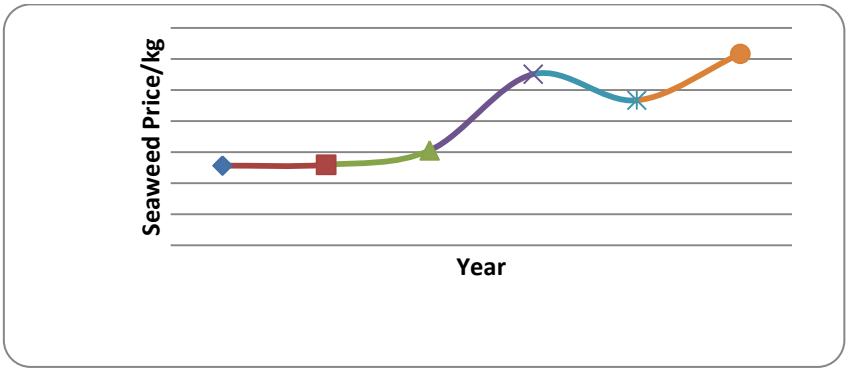


Figure 6 Seaweed price per kilogram in USD Exchange 10.000/US $-Redraw (Cocon, 2013)

Economic Analysis

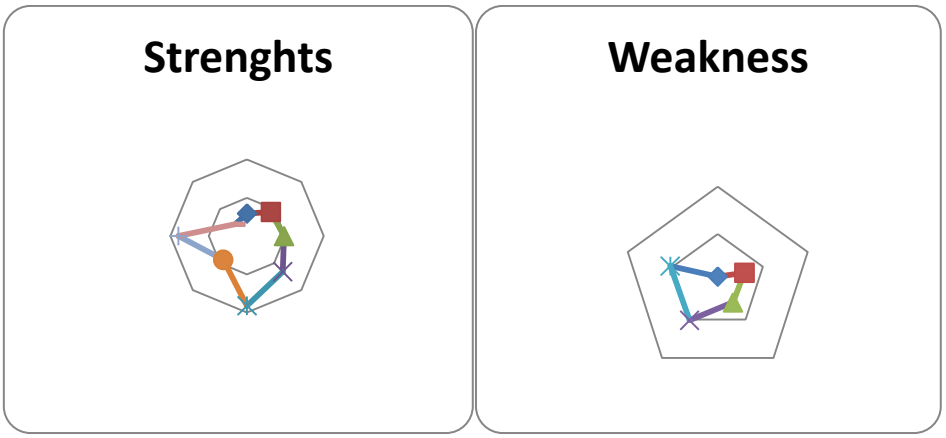
Economic aspects of seaweed cultivation that were analyzed include; level of competition, stability of the global economy, capital, sales and distribution chain. Financial analysis was based on the cash flow of data on Table 2. Furthermore a calculation was also done, based on the criteria of investment which includes NPV (Net Present Value), IRR (Interest Rate Ratio) and PBP (Pay Back Period). The profit of seaweed cultivation in Serang Minapolitan, for over 10 years of investment, is IDR 223.614.298. The results of interest rate analysis obtained a value of 91%. The accumulation of positive NPV values and interest rates greater than 10% of current rates indicate that cultivation of seaweed in Serang Minapolitan is a decent bussiness and gives a positive signal for investors to invest their capital. Through the PBP calculation, it is discovered that capital funds of IDR 60.5 million will return to investors in the span of 1 year and 1 month period of investment.

Tabel 2 Cashflow seaweed farming for 10 years (IDR)

Initial equipment + seed investment		(60.500.000)	
Cash flow year 1	47.830.000	Cash flow year 6	46.221.200
Cash flow year 2	51.330.000	Cash flow year 7	39.142.560
Cash flow year 3	46.174.000	Cash flow year 8	42.642.560
Cash flow year 4	49.674.000	Cash flow year 9	34.763.328
Cash flow year 5	42.721.200	Cash flow year 10	38.263.328

SWOT Analysis

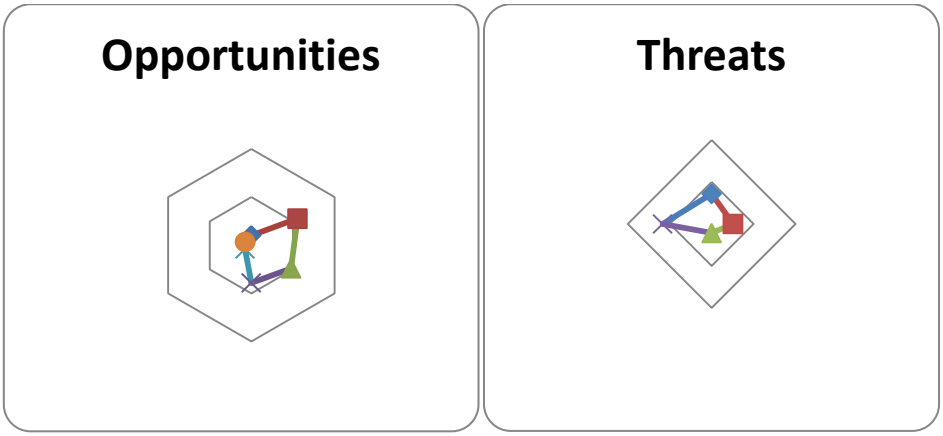
At the phase of SWOT analysis, evaluation of internal and external factors results a mapping of seaweed cultivation conditions in Minapolitan Serang Banten. The strength of bussiness to be analyzed on the availability of land (TWV = 0.91) and the product, is absorbed well by market (TWV = 0.90). However, there is a weakness in the form of seaweed derived products; which have not been produced in a mass number (TWV = 1.05). Farmer groups are also not functioning optimally (TWV = 1.02) (Figure 7).



(a) (b)

Figure 7 Map cultivation of minapolitan *E. cottonii* in Serang. Strengths (a) and Weakness (b)

The mapping of bussiness opportunities and threats are illustrated in Figure 8. The results of analysis opportunities of bussiness were: government policies supporting the cultivation of seaweed were at TWV = 1.11; and the determination of Minapolitan project as a flagship strategic plan were at TWV = 0.96. However, there are threats, in the form of: sale price fluctuation at TWV = 1.17; and global markets affecting the price of seaweed were at TWV = 0.72.



(a) (b)

Figure 8 Map cultivation minapolitan *E.cottonii* in Serang. Opportunities (a) and Threats (b)

Results of evaluation of internal and external factors also result key factors of success. Key factors of success (KFS) is a strategic factor that describes the state of the cultivation of seaweed in the Minapolitan Serang. These factors are considered strategic because they have the highest value of Total Weighted Value (TWV) of any other factor. Key Factors of Success of every factors are shown in Table 3 below:

Table 3 Key factors of success

INTERNAL FACTOR			
TWV	**STRENGHTS (S)**	**TWV**	**WEAKNESS (W)**
0.91	Availability of land for cultivation	1.05	Seaweed derived products have not been mass produced
0.90	Product absorbed well by the market	1.02	Farmer groups are not functioning optimally
EXTERNAL FACTOR			
TWV	**OPPORTUNITIES(O)**	**TWV**	**THREATS (T)**
1.11	Government policy supports the cultivation of seaweed	0.72	Selling prices fluctuate
0.96	Minapolitan project as a flagship strategic plan	1.17	Affect the global market price of seaweed

In addition to generating KFS, the evaluation of external and internal factors produces the map position of the cultivation. Based on the map position (Figure 9), the cultivation of seaweed in the Minapolitan Serang is in the Quadrant III position, with coordinates of (0.74, -2.09). This means that internal factors tend to be weak, while opportunities in external factors dominate. So the focus of the program is to minimize weaknesses and overcome internal obstacles to achieve better market opportunities. Proficiency level program needs to be conducted in order to switch cultivation to Quadrant I, which has a strong internal condition that can be maximized to achieve a great opportunity.

Weakness that dominates the cultivation conditions based on KFS, is seaweed derived products have not been mass-produced and the role of farmer groups have not been functioning optimally. The vulnerability can be minimized through government initiatives in processing products derived from *E.cottonii* and assistance to both farmer groups and independent farmers. Through strengthening the internal factors of bussiness, it is expected to optimize the support of the government to seize market opportunities that exist.

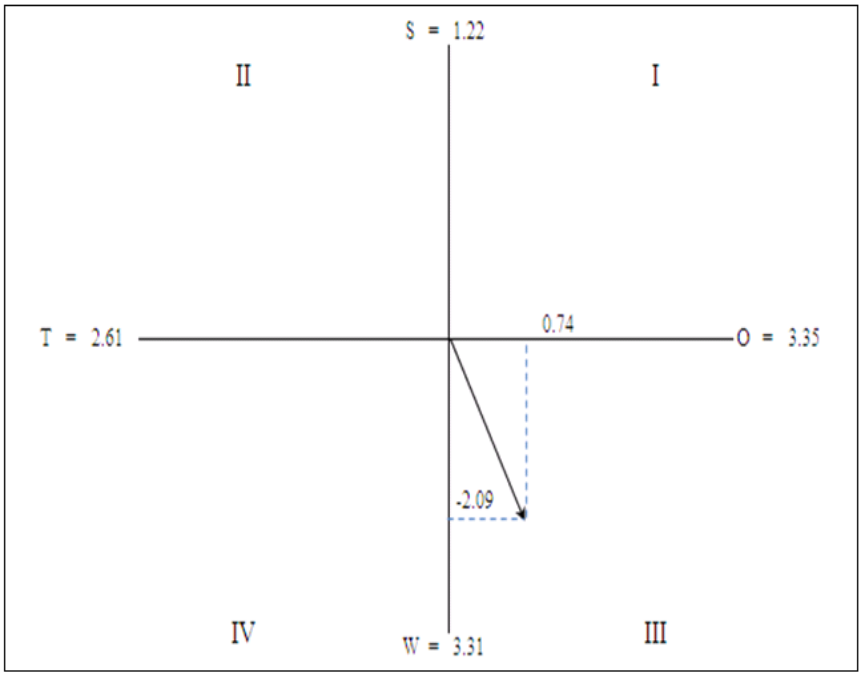


Figure 9 Map of the cultivation bussiness of seaweed in Serang Minapolitan

Preparation of the program can be achieved by integrating the key factors of success to produce the right program. Preparation of the program through SWOT matrix to programs formulation can produce programs that are based on the principles of empowerment leading resources (Sunardi, 2011). Based on the analysis of the map position of previous efforts, the best program is then applied to integrate key factor opportunities (O) and weaknesses (W) toward resolving internal problems, or disadvantages to achieve a better market opportunities. Preparation techniques through SWOT matrix can be seen on Table 4 below.

Table 4 SWOT program formulation for the cultivation of seaweed in Serang Minapolitan

EXTERNAL / INTERNAL	***Threats*** Selling prices fluctuate Affect the global market price of seaweed	***Opportunities*** Government policy supports the cultivation of seaweed Minapolitan project as a flagship strategic plan
Strengths Availability of land for cultivation Product absorbed well by the market	QUADRANT II (PROGRAM S-T) Optimal land use to offset fluctuations in selling prices. Increase in value-added products through processing	QUADRANT I (PROGRAM S-O) Optimization of land use through the addition of infrastructure assistance to the people of coastal aquaculture Government linking farmers with markets
Weakness 1. Seaweed derived products have not been mass produced 2. Farmer groups not functioning optimally	QUADRANT IV (PROGRAM W-T) 1. Sales of seaweed in a state of value-added finished products 2. Farmer groups determine the bargaining power of seaweed prices	QUADRANT III (PROGRAM W-O) 1. Determination of unit processing / processed seaweed industry in the Minapolitan 2. Continuity of government assistance to farmers' groups to produce derivative products

Referring to the above strategic alternatives, it is necessary to quantify the priority of the alternative through filter theory. Based on the criteria of filter theory, quantification of alternative programs is then conducted. Assessment of programs with the highest priority value will be the proposed strategy. Assessment is based on a scale of 1 to 5, set by the researcher. The results of an assessment of alternative programs are shown in Table 5.

Table 5 Filter theory of program WO (Weakness-Opportunities)

No.	Alternative strategies	Effectiveness	Simplicity	Cost	Total	Priority
1	Determination of unit processing / processed seaweed industry in the Minapolitan	4	3	3	10	II
2	Continuity of government assistance to farmers' groups to produce derivative products	4	4	4	12	I

In addition to assessment based on the filter theory, consideration was made based on the conditions of cultivation area (Table 6). Based on the condition of each village, the proposed strategy is focused on:

1. Seaweed production optimization of the Lontar Village.

Efforts to optimize production suggested leads to quality improvement that can be achieved through product certification. Farmers who have certified products have the advantage in pricing in the market. Quality seaweed *E. cottonii* associated with the post-harvest process that requires the availability of clean water. It became one of the inputs to the government to be able to meet the need for clean water. Certified products have a greater chance of being accepted by the market and the industry with favorable prices, especially for farmers.

2. Government assistance for initiation of processing units in Domas Village.

In addition to the distance, the villagers of Domas are historically more focused on the cultivation of brackish water (milk fish). However, some groups of the village of Domas are proactive and have the initiative to develop the desire to cultivate seaweed into value-added derivative products. In addition, the farmers desire to build cooperative seaweed (Bandar Firdaus, pers. Comm.). It has been submitted to the District DKP and it is in the process of preparation. Ongoing process including farmers participation at events and meetings cooperative education between regions. This can be attributed to the higher level of education of farmers. On this basis, the village of Domas has an opportunity to become the location of industrial development / seaweed processing unit that produces a value-added derivative products.

Table 6 Comparison conditions of Domas and Lontar Village

Factor\ Location	**Domas Village**	**Lontar Village**
Potential cultivation	Brackish water	Sea water
Location cultivation	5-7 km water trip	Near settlement
Government initiation	Year 2011	Year 2009
number of farmers	~ 100 person	~ 300 person
Education level of farmers	Elementary school-college	Not in school-elementary school

Conclusion

1. The cultivation of seaweed in Serang is feasible and based on:

- Environmental aspects: environmental conditions of cultivation according to criteria of SNI 7579.2:2010.

- Techniques aspects: use of longline method in accordance with the conditions of substrates waters in the form of sand and sludge alluvial.
- Social aspects: cultivation supported by market opportunities and strong role of government.
- Economic aspects: NPV for the interest rate of 10% during the 10-year effort is IDR 223.614.298. Time refund for 1 year 1 month with a 91% IRR.

2. The proposed strategy to be implemented is based on the map position of Quadrant III enterprises (WO Quadrant: 0.74, -2.09), which minimizes the disadvantages, subsequently achieving a better market. The program priorities are of the following: 1). Continuity of government assistance to farmers' groups to produce derivative products preceded certification of raw material product. Production optimization is proposed in Lontar Village and 2). Determination of unit processing/ processed seaweed industry in the Minapolitan especially in Domas Village.

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We would like to thank Rangga (PT Gumindo Perkasa Indonesia), the Department of Marine and Fisheries of Banten Province and Serang District as well as the Central Regional Research and Development of Banten who have provided information, data, and valuable discussion for this thesis.

References

Cocon, *Status Rumput Laut Indonesia Peluang dan Tantangan*. Slide share. http://www.slideshare.net/070581/status-rumput-laut-indonesia-peluang-dan-tantangan-2, (10 Februari 2013).

Delinom, R.M., *Sumber Daya Air di Wilayah Pesisir dan Pulau-Pulau Kecil di Indonesia,* 1st ed, LIPI Press, 2005.

Denantika, D.P., *Analisis Faktor - Faktor yang Mempengaruhi Ekspor Rumput Laut dan Kajian Trend Volume Ekspor Rumput Laut Indonesia ke China*, Undergraduated thesis, Agribussiness Department, Institut Pertanian Bogor, Bogor, 2012.

Dinas Kelautan dan Perikanan (DKP), *Laporan Akhir Kegiatan Pemantauan Kesehatan Ikan dan Lingkungan Budidaya Dinas Anggaran 2010,* Dinas Kelautan dan Perikanan, Pemda Kabupaten Serang, 2010.

Mustamin, S.F., *Studi Pengaruh Konsentrasi KOH dan Lama Ekstraksi terhadap Karakteristik Karagenan dari Rumput Laut (Eucheuma cottonii),* Undergraduated thesis, Agriculture Department Hasanuddin University, Makassar, 2012.

Purbani, D., Sukresno, B., Mustikasari, E., Kusumah, G. & Solihuddin, T., *Optimalisasi Data Fisik Perairan untuk Kajian Kelimpahan dan Jenis Ikan di Teluk Banten,* Badan Riset Kelautan dan Perikanan, Departemen Kelautan dan Perikanan, 2010.

Rangkuti, F., *Analisis SWOT: Teknik Membedah Kasus Bisnis,*1st ed, Gramedia Pustaka Utama, 2006.

Setyaningsih, H., *Kelayakan Usaha Budidaya Rumput Laut Kappaphycus alvarezii dengan Metode Longline dan Strategi Pengembangannya di Perairan Karimunjawa*, Master thesis, Small-Medium Industry Department, Institut Pertanian Bogor, Bogor, 2011.

Sunardi, S., *Strategi Indonesia dalam menghadapi Liberalisasi Jasa Telekomunikasi dalam Kerangka ASEAN Framework Agreement of Services (AFAS),* Master thesis, Telecommunications Management Department, Indonesia University, Jakarta, 2011.

Tjitroresmi, E., *Potensi dan Pemanfaatan Hasil Sumber Daya Ekonomi Budidaya Rumput Laut*, Optimalisasi Pemanfaatan Sumber Daya Ekonomi Hayati Laut Kasus Budidaya Rumput Laut, Zarmawis Ismail (1st ed), LIPI Press, pp. 17-42, 2009.

Yasita, D. & Rachmawati, I.D., *Optimasi Proses Ekstraksi pada Pembuatan Karaginan dari Rumput Laut Eucheuma cottonii untuk Mencapai Foodgrade,* Undergraduated thesis, Chemical Engineering Department, Diponegoro University, Semarang, 2009.

Storage Temperature and Fungicide Effect on Fruit Quality During Storage Period: A Case Study in PT Mayasari Bakery

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ABSTRACT - Production and export demand for bananas in Indonesia continues to increase. Nevertheless, there is still problem encountered in banana supply chain, that is poor quality due to post-harvest handling imperfections which impact on increasing the number of bananas that can not be consumed. Improvement on banana post harvest management is needed to increase its economic value. This study aimed to acquire banana storage conditions that suitable for keeping the banana quality and longer its shelf life. This study examines the effect of fungicide and storage temperature on banana quality during storage. Bananas are soaked in fungicide before ripening process with calcium carbide, followed by banana storage in three temperature variations, 20°C, 25°C, and 27°C. Based on the research results, banana immersion in fungicide 0.2% gave effect on fungal growth suppression, fungicide effect was obvious in temperature storage of 27°C and 25°C. Bananas in temperature storage of 20°C have the longest shelf life, it can last for 14 days, while bananas at 25°C can last for 11 days, and bananas in 27°C can only last for 7 days. Based on the results, banana immersion in fungicides and fruit storage at 20°C are important to reduce fruit damage and increase fruit shelf life.

Keywords: Banana, Fungicide, Storage temperature, Post-Harvest, Shelf life.

Introduction

Indonesia is one of banana leading producers in the world, after India, Ecuador, Brazil, the Philippines, and China (Suhartanto *et. al.*, 2012). Banana production in Indonesia has increased from 4.300.422 tons in 2001 to 5.037.472 tons in 2006 (Prabawati *et. al.*, 2008). Banana became one of main commodity in Indonesia because it has good market potential both domestic and international. Indonesian banana export volume has increased from 244.652 tons in 2003 to 4.280.641 tons in 2006 (Prabawati *et. al.*, 2008). Nevertheless Indonesia is still importing banana, this is due to banana poor quality. Inability of Indonesian farmers in improving the quality and shelf life of banana causing low selling price, thus it can only enter the local market. Poor handling on banana post-harvest also led to a high number of bananas that can not be consumed, causing economic losses. The main problems faced by banana industry are fruit physical damage which led to early rotting, post-harvest diseases caused by microorganism infection, and mixed ripening.

Banana cultivar Raja Bulu is one of germplasm commodity Indonesia, it means that banana cultivar Raja Bulu is one of domestic genetic diversity in Indonesia (Sumarno, 2002; Somantri *et. al.*, 2005). Banana cultivar Raja Bulu have a promising market opportunity, both for the domestic market and for export, relating to their abundance use in banana processing industry (Prabawati *et. al.*, 2008). The center production of banana cultivar Raja Bulu Banana is in Java Island.

Banana classified as climacteric fruit, because its maturation process associated with increased in respiration and ethylene production (Bouzayen *et. al.*, 2009). The main changes occurred in banana ripening process are the chlorophyll decomposition, starch decomposition, ethylene formation, and increase in respiration rate (Pantastico, 1975; Surendranathan, 2004). During the maturation process, the structure will become soften, both flesh and skin. This is due to pectin depolymerization and starch changes into sugars (Frederick *et. al.*, 1992; Seymour, 1993).

Post-harvest banana does not have the ability to suppress an increase in environmental temperature. The main damage caused by high temperature is cell membrane damages, liquidification of fat or lipids, and nucleic acid and protein denaturation. Visible effects due to high temperature stress are reduction in banana shelf life and quality, and also delay in color changes (Utama & Pratiwi, 2009). Banana is also sensitive to low temperatures. Low temperature storage affects physical properties of cell membrane. Visible damages such as discoloration, failed in maturation, skin and flesh color change to black or dark brown, and changes in fruit taste (John & Marchal, 1995). Damages caused by low temperatures generally occurs below the storage temperature of 13°C. Storage temperature affects respiration and ethylene production, the higher storage temperature, respiration rate and ethylene production will increase (Davis, 2012). Storage temperature affects food decay by interfering chemical reactions. When the storage temperature is high, the rate of food chemical reactions will increase, causing protein denaturation, vitamin destruction, loss of water content, and changes in color, aroma, and taste.

Common diseases that often attack banana are anthracnose, black spot, brown spot, crown rot complex, and scab. In general, the diseases are caused by fungal infection. Anthracnose caused by the fungus *Colletrotrichum musae* et curt V.aryx or *Gleosporium musarium* cice Et massae (Prabawati *et. al.*, 2008), Black Spot (Speckle disease) caused by the fungus *Helminthosporium torulosum* Syd Ashby and Ell *Deigthtoniella torulosa* (Kuntarsih, 2012), Brown spot is caused by the fungus *Cercospora hayi* Caplo (Kuntarsih, 2012), Crown rot complex caused by the fungus *Thielaviopsis paradoxa, Lasiodiplodia theobromae, Colletotrichum musae, Deightoniella torulosa,* and *Fusarium roseum* (Davis, 2012), and Scab is caused by the fungus *Sphaceloma* sp (Kuntarsih, 2012). Fungicide is used to handle the diseases caused by fungal infection. Fungicides are toxic compounds to fungi by damaging cell membranes, inactivation of enzymes and proteins, or interfering the essential processes in the cell such as energy production or respiration (McGrath, 2004). Fungal infections can occur both on the farm and during post-harvest handling. Some fungi are dormant, meaning no damage occurs or

visible at the time of planting, but develops during harvest and maturation. This infection is called latent infection.

Material and Methods

Bananas used in this study are obtained from PT Mayasari Bakery. Treatment of banana includes immersion in fungicide solution 0.2%, followed by ripening process with calcium carbide for ± 1 days, and then stored at 3 various temperature, which are 20°C, 25°C, and 27°C. Experiment carried out in 14 days and duplo. Microorganism and banana physical analysis are observed on day 0, 1, 4, 7, 10, and 14.

Fungal Growth Analysis

Fungal growth analysis is carried out by soaking the banana in NaCl solution 0.85%, followed by making serial dilution. About 0.1 mL of dilution is plated using Total Plate Count method in medium Potato Dextrose Agar (PDA). Fungal colonies are counted after incubation at room temperature for 3-4 days. The number of fungal colonies are counted by units colony forming units per milliliter of sample (CFU/mL).

Banana Physical Appearance Analysis

Analysis of physical appearance aimed to determine the effect of temperature on quality and shelf life of banana cultivar Raja Bulu. Banana color changes are determined at every observation time using banana color index in Table 1.

Result and Discussion

Fungicide Effect

Effect of fungicides can be seen in Table 2. Based on fungal quantification using Total Plate Counting (TPC) in Table 2, it appears that there are more number of fungal colonies in banana without fungicide treatment (-F-K and –F+K). This appears in every variation of temperature, from the first day until day 14. Fungicide (+F +K) gave effect on fungal growth supression, especially visible when the fruit has undergo maturation. In this condition, fruit provides suitable conditions for fungal growth.

Table 1 Banana maturity level based on skin color [2]

Color Index (CI)	Fruit Appearance	Description
1		The entire surface of fruit is green, the structure is hard
2		Light green (breaking toward yellow)
3		Yellowish green
4		Greenish-yellow (more yellow than green)

5		Yellow with green tips
6		Yellow
7		Yellow, flecked with brown
8		Yellow, with many brown flecks

Based on Total Plate Count (TPC) result, the number of fungal colonies on banana in storage temperature of 27°C is greater than the number of fungal colonies on banana at storage temperature of 20°C and 25°C. This is because bananas at storage temperature of 27°C had earlier maturation causing high rate of fungal growth. The number of fungal colonies on banana in storage temperature of 20°C is lower than at temperature of 25°C and 27°C, this is due to delay in maturation and dry physical condition that are not suitable for fungal growth.

Table 2 Effect of Fungicides on Fungal Growth At Storage Temperature of 20°C, 25°C, and 27°C

Time & Temp. / Treatment	T1 (CFU/mL)			T7 (CFU/mL)			T14 (CFU/mL)		
	20°C	25°C	27°C	20°C	25°C	27°C	20°C	25°C	27°C
Control (-) F (-) K	208	357	101	217	4538	3511	215	3987	Spoiled
(-) F (+) K	180	190	424	489	1266	93618	314	130255	Spoiled
(+) F (+) K	108	488	<10	93	447	100878	670	8674	Spoiled

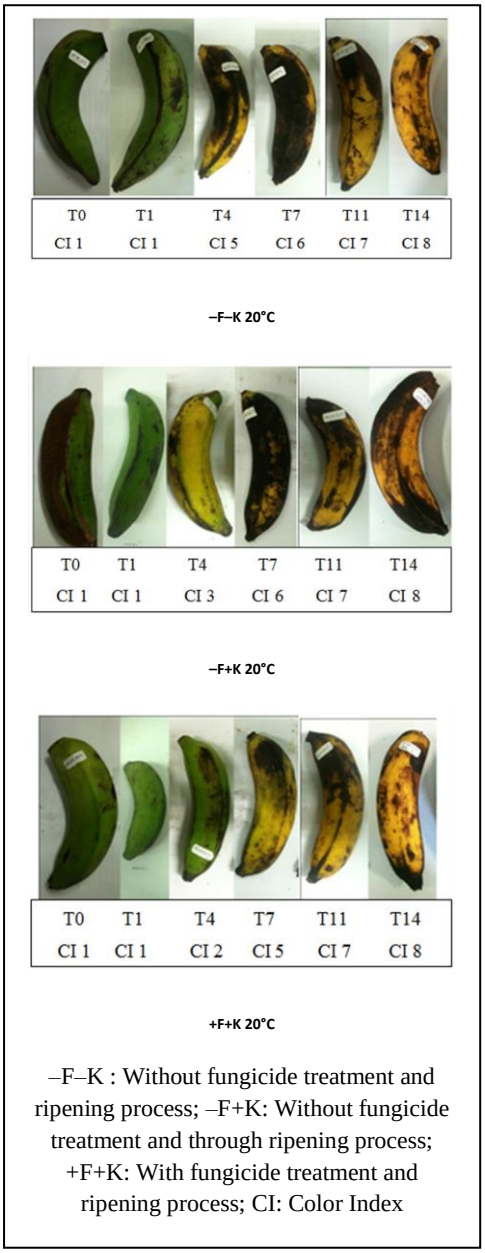
-F-K: Without fungicide treatment and without ripening process; -F+K: Without fungicide treatment and through ripening process; +F+K: With fungicide treatment and through ripening process; Tn: n-time after immersion in fungicide

Based on these results, it can be concluded that the fungicide gives effect on fungal growth supression, especially when the fruit is in ripe condition. Ripening fruit provides optimum conditions for fungal germination and thus fungi can penetrate the skin fruit to reach the fruit flesh. Noted that advanced maturation led to an increase in rate of fungal growth so fungicide effect was no longer significant. Based on TPC result, there are more fungal colonies on banana without fungicide treatment (-F+K) compared to banana with fungicide treatment (+F+K). Bananas that are soaked in fungicide and stored at a temperature of 25°C and 27°C can extend its shelf life for 1 day, while at temperature of 20°C there was no significant difference between banana with fungicide treatment and banana without fungicide treatment, so it does not show an extending shelf life.

Effect of Storage Temperature on Quality and Banana Shelf Life

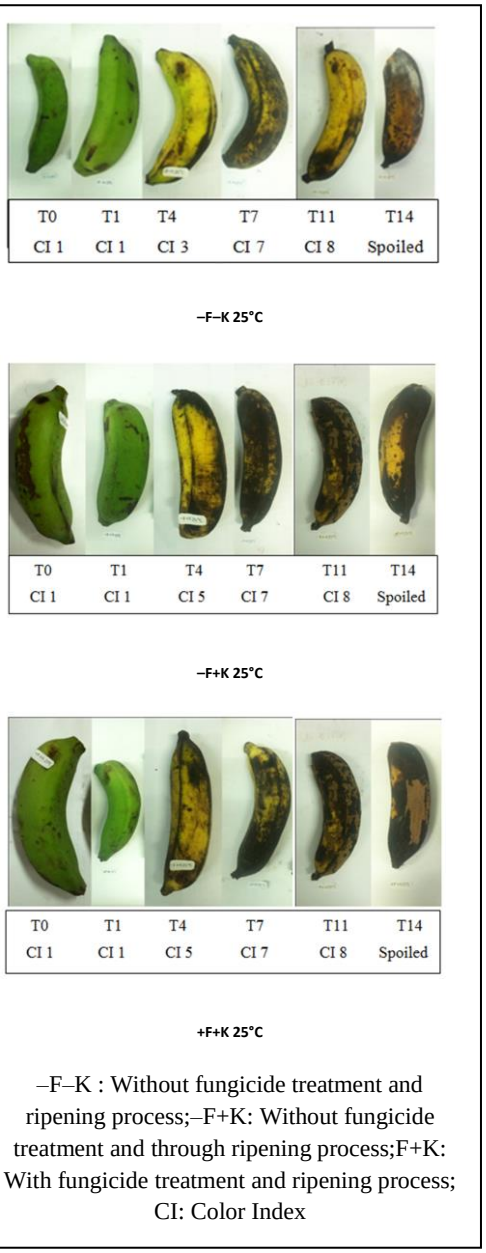
Based on Color Index Table (Table 1), banana at storage temperature of 20°C showed color index 7 (CI = 7) on day 11 and reached CI = 8 on day 14. This can be seen in Figure 1. At the storage temperature of 25°C, banana reach color index 8 (CI = 8) on day 11 of storage. It means that early ripening occurs at temperature of 25°C compared to temperature of 20°C. This can be seen in Figure 1. At the storage temperature of 27°C, banana reach color index 8 (CI = 8) at day 7, indicating decay on day 11. This can be seen in Figure 1.

Based on the result above, it can be seen that storage temperature affects the quality of banana thus affecting banana shelf life by delaying ripening process. Bananas on the storage temperature of 20°C showed drier surface compared to banana at temperature of 25°C and 27°C. Bananas on the storage temperature of 25°C and 27°C showed a damp and wet surface, especially when the fruit undergo maturation, making it suitable for fungal growth and accelerate the decomposition process. Banana delay ripening that occurs at temperature of 20°C is caused by a decrease in respiration rate and ethylene gas formation (Davis, 2012). Banana storage at temperature of 20°C shows double shelf life longer than storage at temperature of 27°C. Banana at storage temperature of 20°C can last for 14 days, while at temperature of 25°C can last for 11 days, and at temperature of 27°C only last for 7 days.



–F–K : Without fungicide treatment and ripening process; –F+K: Without fungicide treatment and through ripening process; +F+K: With fungicide treatment and ripening process; CI: Color Index

Figure 3.1 Banana color index change



–F–K : Without fungicide treatment and ripening process;–F+K: Without fungicide treatment and through ripening process;F+K: With fungicide treatment and ripening process; CI: Color Index

Figure 3.2 Banana color index change

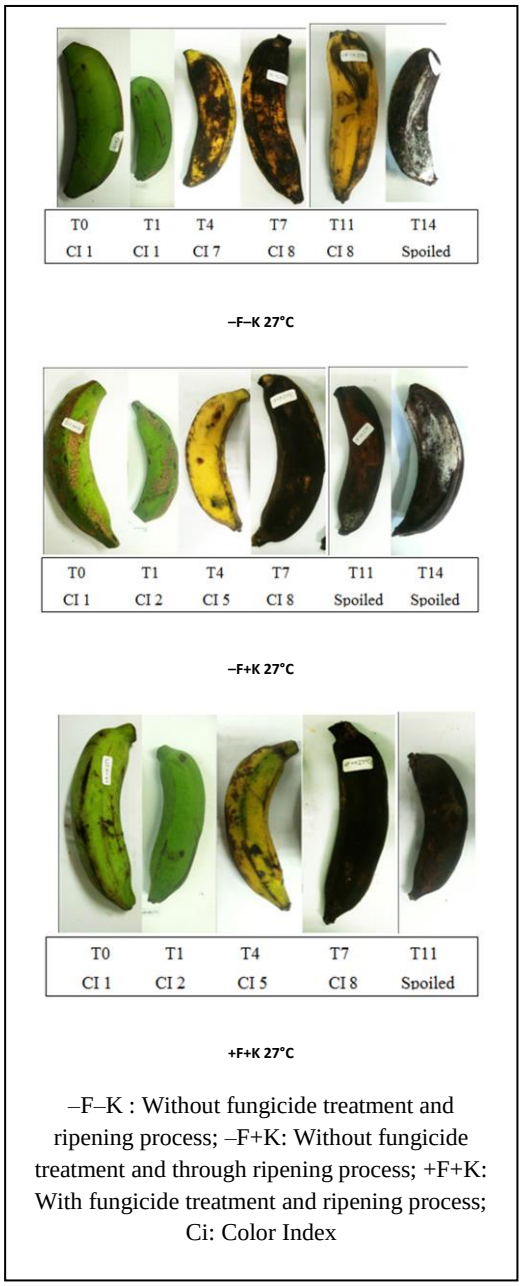


Figure 3.3 Banana color index change at storage temperature of 27°C

Figure 1 Effect of storage temperature to color change of banana

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References

Bouzayen, M., Latche, A., Nath, P., Pech, J.C. *Mechanism of fruit ripening*. *Plant Developmental Biology - Biotechnological Perspectives*, vol 1, 2009.

Davis, U.C. *Banana: Recommendations for Maintaining Postharvest Quality*. Post Harvest Technology University of California. http://postharvest.ucdavis.edu/PFfruits/Banana/ (April 13th 2013)

Frederick B., Morgan, P.W., Mikal, E., Saltveit, Jr. 1992. *Ethylene in plant biology*. California: Academic press Inc., 2012.

John, P., Marchal. *Ripening and biochemistry of the fruit*. In: S. Gowen, ed., Bananas and Plantains, pp. 434-467. Chapman and Hall. London, 1995.

Kuntarsih, S. *Pedoman Penanganan Pascapanen Pisang*. Jakarta, 2012.

McGrath, M.T. *What are Fungicides*. The Plant Health Instructor. DOI: 10.1094/PHI-I-2004-0825-01, 2004.

Pantastico, Er.B. 1975. *Postharvest Physiology handling and utilization of tropical and subtropical fruits and vegetable*. AVI Publishing Company. Westport, Conecticut

Prabawati, S., Suyanti, Dondy, A.S. *Teknologi Pascapanen dan Teknik Pengolahan Buah Pisang*. Balai Besar Penelitian dan Pengembangan Pascapanen Pertanian. Bogor, 2008.

Seymour, G.B. *Banana*. *Biochemistry of Fruit Ripening*. Chapman & Hall, London, pp. 83–106, 1993.

Somantri, I.H., Hasanah, M., Kurniawan, H. *Teknik Konservasi Ex-Situ, Rejuvenasi, Karakterisasi, Evaluasi, Dokumentasi, dan Pemanfaatan Plasma Nutfah*. Balai Penelitian Tanaman Obat dan Rempah, Badan Litbang Pertanian. Bogor, 2005.

Suhartanto, M.R., Harti, H., Haryadi, S.S. *Program Pengembangan Pisang*. Riset Unggulan Berskala Nasional (RUSNAS) Buah Unggulan Indonesia. Jakarta, 2012.

Sumarno. *Penggunaan bioteknologi dalam pemanfaatan dan pelestarian plasma nutfah tumbuhan untuk perakitan varietas unggul*. Seminar Nasional Pemanfaatan dan Pelestarian Plasma Nutfah. Kerjasama Pusat Penelitian Bioteknologi IPB dan KNPN Deptan.Jakarta, 2002.

Surendranathan, K.K. Post-harvest biotechnology of fruits with special reference to banana─perspective and scope. *Indian Journal of Biotechnology* V(4): 39-46, 2004.

Utama, I.M.S., Pratiwi, I.A.R. 2009. *Stress produk pasca panen hortikultura*. Jurusan Teknik Pertanian Universitas Udayana. Bali

FOOD - 06

Analysis of *MaACS2*, a stress-inducible 1-aminocyclopropane-1-carboxylic acid synthase gene in *Musa acuminata* AAA Group cultivar Pisang Ambon

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ABSTRACT - Ethylene plays an important function in plant growth and development. Ethylene production generally increases in response to pathogen attack and other environmental stress condition. The synthesis of this phytohormone is regulated by two enzymes, ACC synthase (ACS) and ACC oxidase (ACO). ACC synthase is encoded by a multigene which regulates the production of ACC, this precursor is then converted into ethylene by ACO. Pisang Ambon (*Musa* sp. AAA group), a banana cultivar originating from Indonesia, has nine ACS genes (*MaACS* 1-9) and one ACO gene (*MaACO*). One of the banana *ACS* genes, *MaACS2*, is stress inducible. In this research, we investigated the expression profile of *MaACS2* in roots and leaves tissues of infected tissue culture plants. The quantification of gene expression was analyzed using Real-Time PCR (qPCR) using *Ma18srRNA* and *MaGAPDH* as reference genes. The results showed nine to ten fold higher *MaACS2* expression levels in infected compared to uninfected roots tissues, however, *MaACS2* expression in the leaves was only detected in infected tissue.

Keywords : banana, environmental stress, ethylene, MaACS2, Real-Time PCR (qPCR)

FULL PAPER ACCEPTED by ITB JOURNAL

Study on The Production of Chitosan from Giant Fresh Water Prawn (*Macrobachium rosenbergii*) Shell from Local Restaurant Waste

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Abstract. Chitosan is a partially deacetylated polymer of N-acetyl glucosamine with degree of deacetylation (DD) as one of the parameter used to state chitosan quality. This research was done to observe the effects of steps on deacetylation process of chitin from giant fresh water prawn shell (*Machrobrachium rosenbergii*) on its degree of deacetylation. Chitosan was made through three stages which were demineralization, deproteinization and deacetylation. Demineralizaion was done using 2N HCl, room temperature, in various steps from 1 to 7 with 30 min for each step. The demineralization steps resulted on decreasing of calcium content from 34.55±5.8% to 0.071±0.07% by 7 steps of HCl changing. The chitin then continued to deacetylation process by using 1:20 (w/v) 50% NaOH, 100^{o}C, with various steps from 1 to 10 step with 1 hour for each step. DD was obtained by using Fourier-transform infrared spectroscopy. Resuts showed that DD 92.75% can be reached by 8 steps. This high DD chitosan could prove particularly suitable for wide range of application especially for medical/analytical applications.

Keywords: *chitosan, degree of deacetilation, giant fresh water prawn shell*

Introduction

Chitin and chitosan are attracting a great deal of attention because of their distinctive biological and physicochemical characteristics as fruit preservation, wound dressings, cosmetics, artificial organs and pharmaceuticals (Tsai *et al.*2002). To date, a lot of research has been done to observe chitin and chitosan from various source like shrimp *Solenocera melontho* (Tsai *et al.* 2002), shrimp *Metapenaeus monoceros* (Laila *et al.* 2010), shrimp *Metapenaeus stebbingi* (Kucukgulmez *et al.* 2012), shrimp *Artemia urmiana* (Tajik *et al.* 2008), crab (Felicity *et al.* 2007), *Portunus sanginolentus L.* (Matheis *et al.* 2012), and other sources like fungi, insects, annelids, molluscs, coelenterata etc. However chitosan is only manufactured from crustaceans (crab and crayfish) primarily because a large amount of the crustacean exoskeleton is available as a by product of food processing and restourant.

Giant fresh water prawn (*Macrobrachium rosenbergii*) is fresh water crustacean potential as chitosan source. Giant fresh water prawn show increase in production and reach total production of 95.5 tones in 2012 only in Yogyakarta Special Province (Anonim). This data shows a great potential to be used as a source of chitin and chitosan.

Chitosan production involves four steps named demineralization (DM), deproteinization (DP), decoloration (DC) and deacetylation (DA). Deacetylation is the crucial steps since the degree of deacetylation (DD) is an important property for chitosan as it affects the physicochemical properties, hence determines its appropriate applications, biodegradibility and immunological activity (Tolaimate *et al.* 2002). Deacetylation process usually done by chemical methods using strong basic like NaOH or by biological methods using enzyme.

This research was done to observe the effect on steps on deacetylation process on degree of deacetylation chitosan produced. This research also observes the demineralization process in order to produce low mineral chitin as chitosan row materials.

Material and Methods

Giant fresh water prawn shells were obtained fresh from local restaurant. The shells then washed, boiled for an hour, dried in cabinet drier 60^{o}C over night and crushed to smaller the size. Chemical reagent used were NaOH (technical grades), HCl 2 N (technical grades) and 0.315 % (v/v) sodium hypochloride solution.

Preparation of chiton

Demineralization

Chitin extraction from giant fresh water prawn shells was carried out by acid treatment using 2 N HCl in room temperature under constant stirring as described by Benhabiles *et al*. (2012). Some preliminary study was done to evaluate the effect of solute : solvent ratio, reaction time and effect on HCl changing on ash removal. Solute : solvent ratio were 1:10, 1:20, 1:30, 1:40 and 1:50; reaction time were 30, 60, 90, 120, 150, 180, 210 min; and steps were 1-7 HCl changing. After the process, samples were neutralized to pH 7 then dried in 50^{o}C for 6 hours.

Deproteinization

Deproteinization was done using samples from previous steps using NaOH 3,5%, 75^{o}C under constant stirring for 2 hours. After that, samples were neutralized to pH 7 and continued to decoloration.

Decoloration

Decoloration process was done using 0.315 % (v/v) sodium hypochloride solution (containing 5.25% available chlorine) 1:15 (w/v) for 15 minutes in room temperature under constant stirring. The samples then dried in 50^{o}C for 6 hours.

Deacetylation

Deacetylation was done using NaOH 50%, 1:20 (w/v) at 100^{o}C. In order to evaluate the effect of steps in NaOH changing on DD of chitosan, the steps were various from 1-10 steps with 1 hours for each step.

Determination of the degree of deacetylation of chitosan

Chitosan and KBr at a ratio of 1 : 100 was mixed well, dried, and then made into a disc. The IR spectrum was measured by FT-IR spectrophotometer (Shimadzu, japan) with a frequency range of 400-4000 cm^{-1}. The methods used for determination the DD of chitosan depended on the base-line of the IR spectrum which are given by Rout [10]. Equation 1 is applied to determine the DD value.

$$DD\ \% = 118.883 - [40.1647 \times (A1655/\ A3450)] \qquad (1)$$

where A1655 is the absorbance of the amide band at 1655 cm^{-1} and A3450 is the absorbance of the O-H band at 3450 cm^{-1}. The factor 40.1647 denotes the value of the ratio of A1655/A3450 for fully N-acetylated chitosan. The number 118.883 was proposed to be related to the baseline (Rout, 2001).

Statistical analysis

All experiments, except for DD value were carried out in triplicate and results were expressed as mean ± s.d. Treatments with significant different were continued to Least Significant Different test.

Results and Discussion

Chitosan was produced from giant fresh water shrimp (*Marcrobrachium rosenbergii*) from local restaurant waste. The shells can be seen in **Figure 1**.

Figure 1 Giant fresh water prawn shells.

Shells obtained were boiled first in order to reduce dirt and proteins contain materials that may still stick to the shells. The proximate analyses on the shells are shown in Table 1.

Table 1 Proximate analysis of giant fresh water prawn shells .

No	Parameter	Percentage
1	Moisture	8.07±0.01
2	Ash	34.55±5.8
3	Protein	31.58±0.04
4	Fat	4.77±0.08
5	Carbohidrates	21.02±0.12*

*carbohydrate were calculated by different

The preliminary study on demineralization process can be seen on **Figure 2 and 3**.

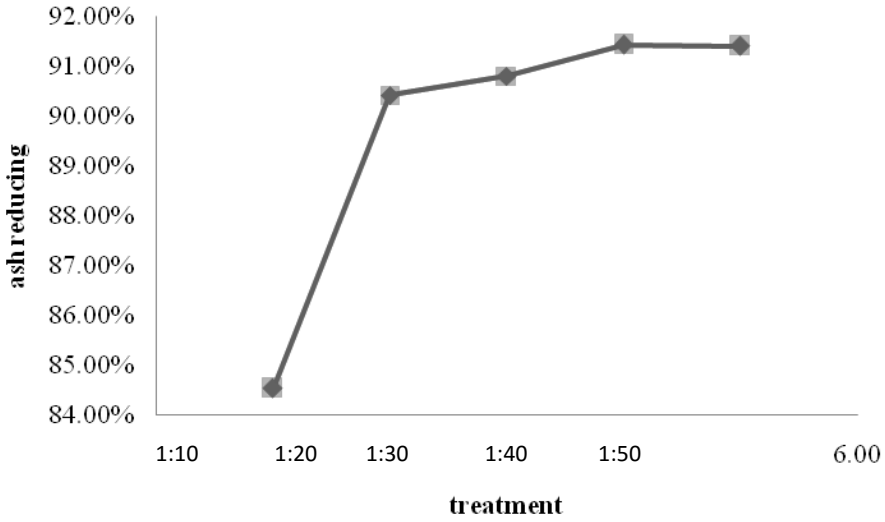

Figure 2 Effect of solute solvent ratio on ash reduction.

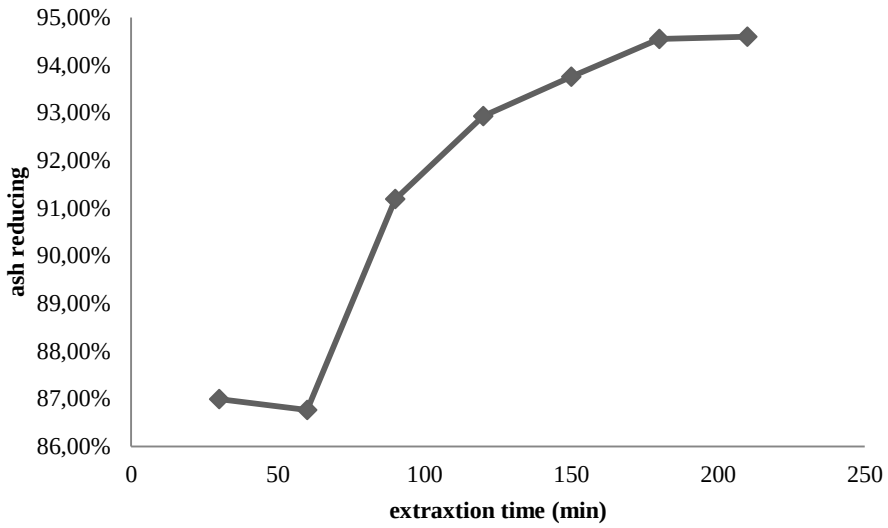

Figure 3 Effect of extraction time on ash reduction.

The preliminary study on the effect of solute solvent ratio resulted on 1:20 as optimum ratio. This ratio able to reduce ash content 90,43±0.07%. On the effect of extraction time, 180 min was the optimum time extraction with 94.55±0.01% on reducing ash content. The ash reduction process is important since ash content able to reduce the protein reduction in the next step. Moreover, the elimination of ash before protein able to produce higher yield and DD value of chitosan. This is explained in the research of Kim (2004) on crawfish chitosan and Tajik *et al*. (2008) on Artemia chitosan.

These results then used in the next study on the effect of the HCl changing steps on the ash reduction. The results is showed in **Figure 4**.

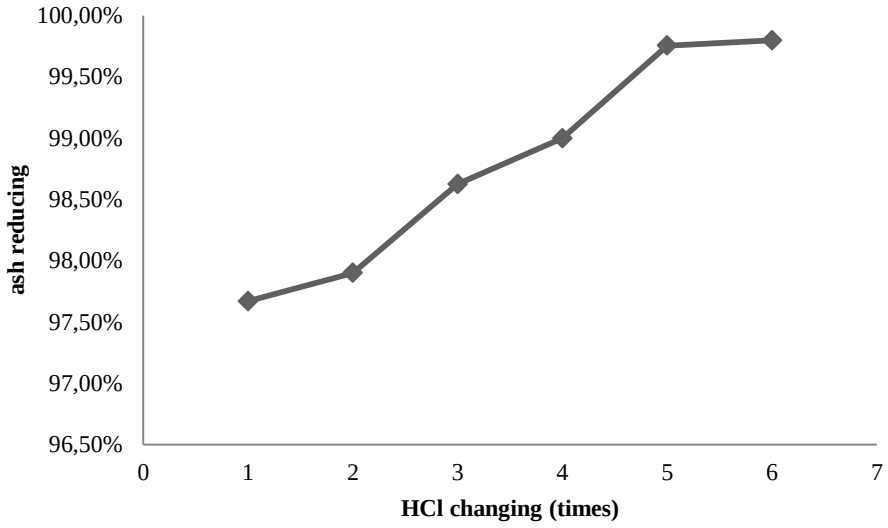

Figure 4 Effect of HCl changing on ash reduction

This result showed that changing HCl 6 times on 180 min extraction able to reduce ash content 99.75±0.006%.

The deacetylation process conducted by varying the steps from 1-10 steps. One step is one hour process using 50% NaOH, 1:20 (w/v) at 100^{o}C. The resutls showed in Table 2.

Table 2 DD value of chitosan.

No	Treatments	DD value (%)
1	1 times	87.94
2	2 times	89.71
3	3 times	91.83
4	4 times	88.52
5	5 times	88.69
6	6 times	89.58
7	7 times	89.49
8	8 times	92.74
9	9 times	92.50
10	10 times	91.22

The DD value of chitosan are varying from 87.94% to 92.74%. The 8 times steps is resulted the highest DD value at 92.74. This results is considered higher then DD chitosan from Puspawati and Simpen (2010) which varying the concentration of NaOH and temperature for 4 hours and resulted on 88,04% DD at 60% NaOH, 120^{o}C.

Conclusion

Demineralization of giant fresh water shrimp shells condition using HCl 2N, with 1:20 w/v solute solvent ratio and 180 min extraction time able to reduce ash content to 99.75±0.006%. Chitosan with 92.74% DD value can be produced with 8 times deacetylation steps process with 50% NaOH at 100^{o}C.

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References

Anonim, Yogyakarta Fisheries Statistic, Ministry of Marine and Fisheries, Indonesia.

Benhabiles, M.S., Salah, R., Lounici, H., Drouiche, N., Goosen, M.F.A., Mameri, N. Antibacterial Activity of Chitin, Chitosan and Its Oligomers Prepared from Shrimp Shell Waste. *Food Hydrocolloids* 29: 48-56, 2012.

Felicity, B., Clifford, L., Michael, A., Oghenekome, O. Extraction and Evaluation of Chitosan from Crab Exoskeleton as a Seed Fungicide and Plant Growth Enhancer. *American-Eurasian Journal of Agricultural & Environmental Sciences* 2 (2): 103-111, 2007.

Kim, S.O.F. *Physicochemical and Functional Properties of Crawfish Chitosan as Affected by Different Processing Protocols*. [Theses] Lousiana State University, 2004.

Kucukgulmez, A., Gulnaz, O., Celik, M., Yanar, Y., Kadak, A.E., Gercek, G. Antimicrobial Activity of the Chitosan Extracted from *Metapenaeus stebbingi* Shell Wastes. *Journal of Polymers and the Environment* 20:431-437, 2012.

Laila, M., Olfa, G.B., Kemel, J., Islem, Y., Moncef, N. Extraction and Characterization of Chitin, Chitosan, and Protein Hydrolysates Prepared from Shrimp Waste by Treatment with Crude Protease from Bacillus cereus SV1. *Applied Biochemistry and Biotechnology* 162:345–357, 2010

Matheis, F.J.D.P. Tanasale, Amos, K., Marsela, S.L. Kitosan dari Limbah Kulit Kepiting Rajungan (*Portunus sanginolentus* L.) sebagai Adsorben Zat Warna Biru Metilena. *Jurnal Natur Indonesia* 14(2): 165-171, 2012.

Puspawati, N.M., Simpen, I.N. Optimasi Deasetilasi Khitin Dari Kulit Udang dan Cangkang Kepiting Limbah Restoran Seafood Menjadi Khitosan Melalui Variasi Konsentrasi NaOH. *Jurnal Kimia* 1: 79-90, 2010.

Rout, S.K., Ph. D. Thesis on physicochemical functional and spectroscopic analysis of crawfish chitin and chitosan affected by process modification. [Dissertation]. Louisiana State University, Baton, Rouge, La, USA, 2001.

Tajik, H., Mehran, M.M., Seyed, M.R.R., Amir, M.M.F., Farnood, S.S.J. Preparation of Chitosan from Brine Shrimp (*Artemia urmiana*) Cyst Shells and Effects of Different Chemical Processing Sequences on the Physicochemical and Functional Properties of the Product. *Molecules* 13: 1263-1274, 2008.

Tolaimate, A., Desbrieres, J., Rhazi, M., Alagui, A., Vicendon, M., Vottero, P. On the Influence of Deacetylation Process on the Physicochemical Characteristics of Chitosan from Squid Chitin. *Polymer Journal* 41: 2463-2469, 2002.

Tsai, G.J., Su, W.H., Chen, H.C., Pan, C.L. Antimicrobial Activity of Shrimp Chitin and Chitosan from different Treatments and Applications of Fish Preservation. *Fisheries Science* 68: 170–177, 2002.

FOOD - 08

Promoting Dolichoderus thoracicus as an agent to disperse Trichoderma sp, a fungi that control the black pod disease, in Center of Sulawesi - Indonesia

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ABSTRACT - In this study, we propose to use *Dolichoderus thoracicus* to act as a double agent, not only as an agent to control cocoa plant pests, *Conopomorpha cramerella* and *Helopeltis theobroma*, but also as an agent in distributing *Trichoderma* sp., a fungus that suppress the development of the black pod disease caused by *Phytophthora palmivora*. Selected *Trichoderma* sp. were grown in several pretreated soil and the result showed that *D. thoracicus* were more attracted to honey-soil media (M5) and coconut waste pulp-soil media (M6), however, 10 % sucrose-potato-soil media (M3) was the best media for growing *Trichoderma* sp. therefore more *Trichoderma* spores (87.43×10^2 cfu/individu) were carried by these ants. Morphological study using microscope with 400x magnification showed that the spores attached to legs, antennas, head, thorax, and abdomen of *D. thoracicus*. The efficiency of *D. thoracicus* in distributing the spores compared to the conventional one using a sprayer, was measured by calculating the percentage of *P. palmivora* growth suppression; and the result showed that the growth of *P. palmivora* was suppressed by 83.33% which was not significantly different with that when the *Trichoderma* was disperse through spraying (87% suppressed). As there was a reduction in the growth of *P. palmivora*, the black pod disease in cocoa fruit was also declining. From this study, it was concluded that *D. thoracicus* can act as a double agent and used as an agent to disperse *Trichoderma* sp.

Keywords : *Black pod disease, D. thoracicus, P. palmivora, Trichoderma* sp.

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FOOD - 09

Improvement of Cavendish Banana Embryo Cultures (*Musa acuminata colla* (AAA Group)) Using Transformation Mediated by *Agrobacterium tumefaciens* Strain GV3101/pBI121

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ABSTRACT - Genetic transformation mediated by *Agrobacterium tumefaciens* is one of common method to enhance the quality of plant, which can also be applied to Cavendish banana fruit with polyploidy genome (*Musa acuminata colla* (AAA Group)). Therefore, the establishment of transformation procedure mediated by *A. tumefaciens* in Cavendish banana embryo in solid cultures has been conducted. Male-flower-derived embryo cultures and *A. tumefaciens* strain GV3101, harboring pBI121 binary vectors carrying *nptII* (*neomycin phosphotransferaseII*) and *gusA* (*β-glucuronidase*) in the T-DNA, were used to investigate T-DNA delivery into tissue culture and to evaluate the expression of *nptII* and also *gusA* in banana embryo after being transformed. Seven days Cavendish banana embryo cultures were inoculated by *A .tumefaciens* using submerge method for 30 minutes. Co-cultivation was conducted in vitro, in CCM semisolid medium added with 100µM *acetosyringone* and 0,02% *pluronic F68*. After 3 days of co-cultivation, the expression of *nptII* and *gusA* in explants was tested. Histochemical GUS assay in putatively transformed embryos demonstrated expression of *gusA*.

Keywords: *Agrobacterium tumefaciens*, banana embryo cultures, gusA, GV3101, nptII, pBI121.

FULL PAPER WITHDRAWED by AUTHOR

Dynamics of Cocoa Beans' Pulp Degradation during Cocoa Bean Fermentation: Effects of Yeast Starter Culture Addition

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Abstract. Cocoa beans fermentation is a crucial step in the post-harvest processing of cocoa beans. This process comprised of mix culture microbial activities on the cocoa bean pulp, producing some metabolites that act as important precursors for cocoa flavour development. Variations on microbial population dynamics during the fermentation process may induce change in the overall process and thus the introduction of specific microbial starter may improve the quality of the fermentation. This article discussed the effects of the addition of *Saccharomyces cerevisae* var. Chevalieri starter culture to the cocoa bean fermentation. Followingly, dynamics in yeast concentration, sugary pulp components and metabolic products were measured. Although only a slight difference was observed in the yeast population during the fermentation, the changes in the dynamic metabolite profile were significant and by the end of experiments higher fermentation index was measured for the cocoa bean fermentation with yeast starter culture addition. Our results showed that this method could potentially be applied to shorten the cocoa bean fermentation time.

Keywords: yeast; starter; cocoa bean; fermentation; pulp degradation.

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FOOD - 11

Chitinase (*MaChi*) Gene from Indonesian Banana Plant: Isolation, Characterization, and the Use as Molecular Marker for Disease Resistance

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ABSTRACT - Chitinase is an enzyme produced by plants as a defense protein against pathogens. Fragments of putative chitinase gene (*MaChi*) were isolated from genomic DNA of five Indonesian banana cultivars (Rejang, Klutuk Wulung, Kepok, Ambon Hijau and Barangan).The fragments were characterized and used to develop single nucleotide amplified polymorphisms (SNAP) markers for disease resistance. Fragments of *MaChi* gene containing three exons (a total of 438 bp) and two introns (158 bp) were identified. From these, nucleotide sequence variabilities were identified among eight unique sequence identities. Based on the results of nucleotide sequence analysis, the *MaChi* fragments contained a typical glycoside hydrolase conserved region of chitinase family 19 and exhibited a high sequence identity to either class I or class II chitinase. The result of sequence analysis also indicated the presence of 14 SNP positions causing 11 amino acid substitutions. The presence of SNPs in the *MaChi* gene may be used to generate SNP-based marker for disease tolerance.

Keywords: chitinase, banana, single nucleotide polymorphism, marker.

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FOOD - 12

Expression Study of *LeGAPDH*, *LeACO*1, *LeACS*1A, and *LeACS*2 In Tomato Fruit (*Solanum lycopersicum*) for Future Agroindustry Application

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ABSTRACT - Tomato is a climacteric fruit, which is characterized by ripening-related increase of respiration and elevated ethylene synthesis. Ethylene is the key-hormone in ripening process of climacteric fruits. The objective of this research is to study the expression of three ethylene sythesis genes; *LeACO*1, *LeACS*1A, *LeACS*2, and a housekeeping gene *LeGAPDH* in ripening tomato fruit. Specific primers have been designed to amplify cDNA fragment of *LeGAPDH* (143 bp), *LeACO*1 (240 bp), *LeACS*1A (169 bp), and *LeACS*2 (148 bp)using polymerase chain reaction (PCR). Nucleotide BLAST results of the cDNA fragments show high similarity with *LeGAPDH* (NM_001247874.1), *LeACO*1 (NM_001247095.1), *LeACS*1A (NM_001246993.1), *LeACS*2 (NM_001247249.1), respectively. Expression study showed that *LeACO*1, *LeACS*1A, *LeACS*2, and *LeGAPDH* genes were expressed in ripening tomato fruit. Isolation methods, reference sequences, and primers used in this study can be used in future experiments to study expression of genes responsible for ethylene synthesis using quantitative PCR and to design better strategy for controlling fruit ripening in agroindustry.

Keywords: Ripening, tomato fruit, *LeACS*1A, *LeACS*2, *LeACO*1, *LeGAPDH*

FULL PAPER ACCEPTED by HAYATI JOURNAL

Optimization of Fermented Tofu with High Isoflavone Content through Variation of Percentages and Inoculum Ratios of *Lactobacillus plantarum, Lactobacillus acidophilus,* and *Leuconostoc mesenteroides*

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ABSTRACT - Tofu is a traditional food from Indonesia. Tofu can be made through fermentation using lactic acid bacteria. Lactic acid bacteria had an important role in producing taste, flavor, texture, and color of fermented food. It also can produce isoflavone which has activity against cancer and cardiovascular diseases. In this research, standardization process was done using variation of percentages and ratios of *Lactobacillus plantarum* (a), *Lactobacillus acidophilus* (b), and *Leuconostoc mesenteroides* (c). The percentages of inoculum used in this research was 7.5%, 10%, and 12.5%, meanwhile the ratios of inoculum used was (a:b:c) 2:1:1; 1:2:1; 1:1:2; and 1:1:1. Optimum inoculum age of *L. plantarum, L. acidophilus,* and *L. mesenteroides* respectively were 8, 6, and 2 hours. The highest growth rate of *L. plantarum, L. acidophilus,* and *L. mesenteroides* respectively were $0.060h^{-1}$, $0.054h^{-1}$, and $0.092h^{-1}$. The highest lactic acid production rate of *L. plantarum, L. acidophilus,* and *L. mesenteroides* respectively were 0.072 $\%h^{-1}$; 0.063 $\%h^{-1}$; and 0.126 $\%h^{-1}$. The percentage and ratio of the inoculum which produce tofu with the highest isoflavones content was 12.5% (2:1:1), with the highest bacterial population growth rate was 0.019 h^{-1}; the highest formation rate of lactic acid was 0.045% h^{-1}; and isoflavone aglycones levels was 0.445 mg/mL.

Keywords: fermentation, lactic acid, lactic acid bacteria, isoflavone, tofu.

FULL PAPER ACCEPTED by ITB JOURNAL

Application of Clay Pot as Post Harvest Storage for Tomato

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ABSTRACT - In Indonesia, tomato is considered as an important economical crop. However, most of harvest ended up being wasted due to lack of proper post harvest processing technology. Since most of the Indonesian farmers are considered as low-income rural farmers and have limited access to reliable power supply the development of a low cost and low energy demand storage system is needed. This research applies the concept of evaporative cooling using clay pot filled with water soaked sand as storage system for fresh tomato. The effectiveness of this system to maintain fruit quality (weight loss, water content, and fruit sweetness) was compared with common domestic refrigerator. On average, this system has lower temperature than room temperature with average temperature between 21-23^{o}C. Tomato stored inside clay pot filled with lowest water soaked sand (sand volume 500 g) has lowest weight loss (3.01%),lowest water content (94%), highest fruit sweetness (4.675 brix). Further research on various regions with different environment condition and measurement on other fruit quality variables, efficiency, and economic benefit is needed prior to its application on rural farming.

Keywords: Clay pot, Evaporative cooling, fruit quality, post harvest storage, tomato

Introduction

In Indonesia, despite being perfectly capable of producing abundant harvests, without any means of store and preserve crops, Indonesian farmers are at risk for heavy economic loss due. One of the crops that experience heavy loss of harvest is tomato (*Lycopersicon esculentum*). Annually, total tomato production is 853,061 ton (Statistik Indonesia, 2009) and in tropics estimate about 20-50% of production is loss due to poor post harvest handling (Prajawati, 2006).

Common application to prevent harvest lost of tomato is cool storage (Aguayo *et. al.*, 2004) at 10-15^{o}C with average humidity 85-95% (Shewfelfat, 1986; De Castro *et. al.*, 2005). In order to achieve this condition, farmers require specific machinery, such as domestic refrigerator, which quite expensive and inefficient for farmers with low income and access to epileptic power supply. Furthermore, tomato stored under this condition for a long period is susceptible to chilling injury which reduce quality and price of the products (Olosunde *et. al.*, 2009). Thus, in order to improve income of low income farmer, FAO (1983) advocated a low cost storage system based on principle of evaporative cooling for storage of harvested fruits and vegetables. Evaporative cooling is working on the concept of increased relative humidity of storage environment (or decreasing the vapor pressure deficit) between fruits and its environment). Increased relative humidity will reduce the rate of water loss (Katsoulas *et. al.*, 2001) which in advance will inhibit respiratory processes and activities of destructive micro-organisms (Barre *et. al.*, 1988).

Various design evaporative coolers have been reported (Redulla, 1984; FAO, 1986; Roy, 1994; Acedo, 1997) yet most of them either using expensive material, susceptible to rodent attack, or has large size that required enormous space.In order to overcome these problems, some researcher tried different approach using clay pot such as a porous wall (pot in pot) (Anyanwu, 2004) and cuboids clay pot (Ndukwu, 2011). The benefit of using clay pots are: they easily developed using local resources, does not need any electricity, and could encourage the development of local clay pot industry.

This study investigatedthe effectiveness of an evaporated cooler system construct with local clay pot to maintain quality of local tomato. Tomato quality (weight loss, water content, and sweetness) was evaluated as a function of storage time for 20 days from the harvesting time.

Material and Methods

Description of the Evaporative Cooling System

The evaporative cooler is made up of 5 liter clay pot, purchase from local clay pot industry, filled with water soaked sand. Sand used in this study was common sand apply for building construction. During this research various proportion of water and sand were applied, as evaporative cooling system,in order to find perfect combination for the cooler (Table 1). Each combination was replicated three times and domestic refrigerator was applied as control treatment.

Table 3 Summary of combination of water and sand used in the research

Group	Water Volume (ml)	Sand Weight (g)	Group	Water Volume (ml)	Sand Weight (g)
E			Control		
A1	40	500	C1	40	1500
A2	80	500	C2	80	1500
A3	120	500	C3	120	1500
A4	160	500	C4	160	1500
B1	40	1000	D1	40	2000
B2	80	1000	D2	80	2000
B3	120	1000	D3	120	2000
B4	160	1000	D4	160	2000

Experimental Methods

During this study, six clay pots were used for each experimental group. Inside each pots, 12 tomatoes, previously wash by tap water, were kept on water soaked sand layer. All pots were tightly sealed by plastic sheet and kept in room temperature for 20 days. Every two days, temperature inside clay pots and fruit quality variables was measured.

Fruit quality

Weight Loss

Weight loss was expressed as a percentage of difference in weight between tomatoes that had been kept for 20 days and the initial weight of tomatoes. The weight of fruits was recorded to an accuracy of ± 0.1 g (Žnidarčič *et.al.*, 2010).

Water content

Water content of tomatoes was measured by gravimetric test. Fresh tomatoes were heated by oven at 105^{o}C for 6 hours. Water content was expressed as percentage of weight loss after treatment (Musaddad, 2002).

Fruit Sweetness

Fruit sweetness was measured using refractrometer digital ATAGO®. Sweetness was expressed as brix value.

Statistical Analysis

The data obtained from this investigation were analyzed by One Way ANOVA with confidence level of 95%. Significant value then became subject of further analysis by Tukey. All analysis was carried out using STATISTICA 7.0.

Results and Discussion

Temperature changes inside clay pot

On average, temperature inside clay pot was lower 2.066 ^{o}C than room temperature (Figure 1). Among all treatments, clay pot filled with lowest total volume of water soaked sand (A1-A4) has significantly higher temperature differences (-2.133 ^{o}C) compare with treatment B1-B4 (-2.075^{o}C), D1-D4 (-2.047^{o}C), and C1-C4 (-2.00 ^{o}C) (*One way ANOVA*, P<0.05).

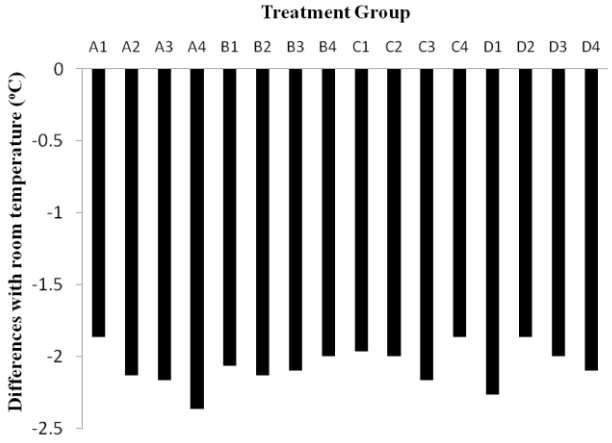

Figure 5 Average temperature differences between inside clay pot and room temperature. Average daily room temperature was 23-25^{o}C.

Lower temperature inside clay pot worked based on principle of evaporative cooling whereby liquid evaporates into air, releasing latent heat, and cooling the object that the air comes into contact with (Shama *et. al.*, 2011). This study showed highest temperature differences when less volume of sand were used. This phenomenon could be explained by concept of thermodynamic and gas movement as bigger empty space reduces collision among gas molecules thus the amount of heat produces.

Fruit Quality

Weight Loss

Storing tomato in clay pot filled with least volume of water soaked sand better in maintained weight of tomato than clay pot with higher volume of sand and domestic refrigerator (*One Way ANOVA*, $P < 0.05$)(Fig. 2).On average, weight loss experienced by tomato stored inside type A system (3.01%) were less than acceptable maximum weight loss for tomato, which is 6-7% (Robinson, 1975; Hruschka, 1977). This result showed that this system able to prevent weight loss even though temperature inside clay pot much higher than temperature suggested for tomato post harvest storage, which is 5-15^{o}C (Shewfelfat, 1986; De Castro *et. al.*, 2009; Žnidarčič & Požrl, 2006).

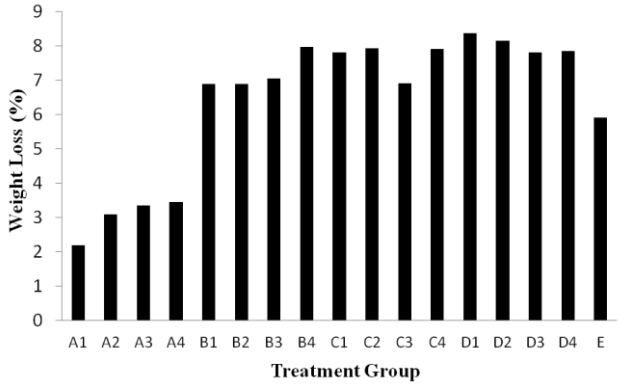

Figure 6 Average weight loss of tomatoes kept in clay pots for 20 days.

Water content

Tomato kept inside clay pot with least volume of water soaked sand has lowest water content compare with all treatment and control (Fig. 3). Lower water content probably correlated with changes in permeability of tomato fruit cuticles due to effect of temperature and relative humidity (Matas *et. al.*, 2005). Further study is needed to confirm about this since loss in water content influenced the shelf life and toughness of the fruit which is crucial for long distance transport.

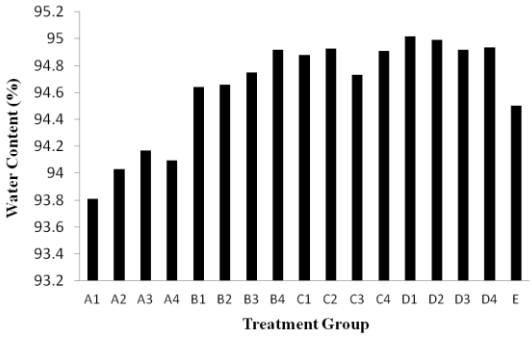

Figure 7 Average water content of tomatoes kept in clay pots for 20 days.

Fruit Sweetness

Tomatoes stored inside clay pot had higher brix level compare with tomatoes stored inside domestic refrigerator. Among all treatment, tomatoes kept inside clay pot with lowest amount of water soaked sand had highest brix level.

Low brix level of tomatoes stored inside refrigerator because sugar converted into starch in low temperature (Adegoroye *et. al.*, 1989; McDonald *et. al.*, 1999). Low brix level also indicated low soluble solids content of fruit, mainly sugar and acid, which would greatly effect to flavor of tomatoes (Moretti *et. al.*, 1998).

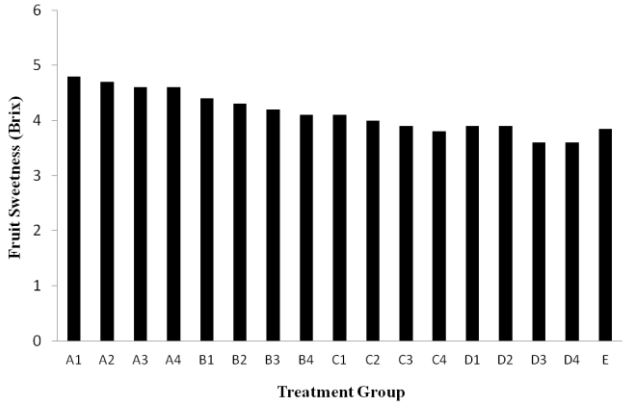

Figure 8 Average fruit swetness of tomatoes kept in clay pots for 20 days.

Conclusion

Clay pot filled with water soaked sand could be used as low cost post harvest storage system for tomato. This system is much better on preventing weight loss and maintaining fruit flavor compare with domestic refrigerator. Further study is needed to confirm the maximum shelf life of fruit, efficiency, and economic benefit of the system.

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References

Acedo, A.L. Storage Life of Vegetables in Simple Evaporative Coolers. *Tropical Science* 37: 169-175, 1997.

Adegoroye, A.S., Jolliffe, P.A., Tung, M.A. Texture Characteristics of Tomato Fruits (*Lycopersicon esculentum*) affected by sunscald. *Journal* of the *Science* of *Food* and *Agriculture*, 49(1): 95-102, 1989.

Aguayo, E., Escalona, V., Artes, F., Quality of Fresh-Cut Tomato as Affected by Type of Cut, Packaging, Temperature and Storage Time. *European Food Research and Technology* 219(5): 492-499, 2004.

Anyanwu, E.E. Design and Measured Performance of a Porous Evaporative Cooler for Preservation of Fruits and Vegetables. *Energy Conversion and Management* 45(13-14): 2187–2195, 2004.

Barre, H.J., Sammet, L.L., Nelson, G.L. *Environmental and Functional Engineering of Agricultural Buildings*, Van Nostrand Reinhold Company, New York, 1988.

De Castro, L.R., Vigneault, C., Charles, M.T., Cortez, L.A.B. Effect of Cooling Delay and Cold-Chain Breakage on 'Santa Clara' Tomato. *Journal of Food Agriculture and Environment* 3(1): 46-54, 2005.

FAO. FAO production yearbook, vol. 34. FAO, Rome, 1983.

FAO. *Improvement of Post-Harvest Fresh Fruits and Vegetables Handling - a Manual*. Bangkok, UNFAO regional office for Asia and the Pacific, 1986.

Hruschka, H.W. *Postharvest Weight Loss and Shrivel in Five Fruits and Five Vegetables*, US. Departement of Agriculture Marketing Research Report 1059, 1977.

Katsoulas, N., Baille, A., Kittas, C. Effect of Misting on Transpiration and Conductance of a Greenhouse Rose Canopy. *Agricultural and Forest Meteorology* 106(3): 233–247, 2001.

Matas, A.J., López-Casado, G., Cuartero, J., Heredia, A. Relative Humidity and Temperature Modify the Mechanical Properties of Isolated Tomato Fruit Cuticles. *American Journal of Botany* 92(3): 462-468, 2005.

McDonald, R.E., Mccollum, T.G. & Baldwin, E.A. Temperature of Water Treatments Influences Tomato Fruit Quality Following Low Temperature Storage. *Postharvest Biology and Technology* 16(2): 147-155, 1999.

Moretti, C.L., Sargent, S.A., Huber, D.J., Calbo, A.G., Puschmann, R. Chemical Composition and Physical Properties of Pericarp, Locule and, Placental Tissues of Tomatoes with Internal Bruising. *Journal of the American Society for Horticultural Science* 123(4): 656-660, 1998.

Musaddad, D. *Mempelajari Efektifitas Pelapis Edible Khitosan pada Buah Tomat Segar Selama Penyimpanan di Suhu Kamar dan Suhu Dingin* (*Study on Efficiency of Edible Chitosan as Coating on Fresh Tomatoes During Room and Cool Storage*). [Magister Thesis] Bogor: Institut Pertanian Bogor, 2002. (In Indonesian).

Ndukwu. M.C. Development of a Low Cost Mud Evaporative Cooler for Preservation of Fruits and Vegetables. *Agricultural Engineering International: CIGR Journal*, Manuscript No.1781, 13(1), 2011.

Olosunde, W.A., Igbeka, J.C., Olurin, T.O. Performance Evaluation of Absorbent Materials in Evaporative Cooling System for Storage of Fruits and Vegetables. *International Journal of Food Engineering* 5(3): 1-15, 2009.

Prajawati N.M., *Pengaruh Teknik Pengemasan dan Perlakuan Prakemas Terhadap Laju Penurunan Mutu Tomat Selama Transportasi* (*Effect of Packaging Technique and Pre Packaging Treatment on Tomato Quality Reduction During Transportation*). [Skripsi]. Bogor: FakultasTeknologi Pertanian,Institut Pertanian Bogor, Bogor, 2006.

Redulla. Temperature and Relative Humidity in Two Types of Evaporative Coolers. *Postharvest Research Notes* 1: 25-28, 1984.

Robinson, J.E., Browne, K.M., Burton, W.G. Storage Characteristics of Some Vegetables and Soft Fruits. *Annals of Applied Biology* 81(3): 399-408, 1975.

Roy S.K. *A Low Cost Cool Chamber: An Innovative Technology for Developing Countries (Tropical Fruits Storage)* in Johnson GI, (Commonwealth Scientific and Industrial Research Organisation, St Lucia (Australia). Division of Horticulture), Editor.Postharvest handling of tropical fruits. Canberra, A.C.T., Australia: Australian Centre for International Agricultural Research 1994. p. 393–395, 1989.

Sharma, V.K., Marano, D., Anyanwu, C.N., Okonkwo, G.I., Ibeto, C.N., Eze, I.S. *Solar Cooling: A Potential Option for Energy Saving and Abatement of Greenhouse Gas Emissions in Africa*, Singapore Journal of Scientific Research 1(1): 1-12, 2011.

Shewfelfat, R.L. Postharvest Treatment for Extending the Shelf Life Fruits and Vegetables. *Food Technology* 40: 70-80, 1986.

Statistics Indonesia, *Produksi Tanaman Sayuran di Indonesia* (*Vegetables Production in Indonesia*), Statistics Indonesia, Jakarta, 2009 (in Indonesian).

Žnidarčič, D. & Požrl, T., Comparative Study of Quality Changes in Tomato cv. 'Malike' (*Lycopersicon esculentum* Mill.) Whilst Stored at Different Temperatures. *Acta Agriculturae Slovenica* 87: 235-243, 2006.

Žnidarčič, D., Ban, D., Oplanić, M., Karić, L., Požrl, T. Influence of Postharvest Temperatures on Physiochemical Quality of Tomatoes (*Lycopersicon esculentum Mill.*). *Journal of Food, Agriculture & Environment* 8(1): 21-25, 2010.

The Study of Pisang Raja Bulu (*Musa* sp. AAB group) *MaACS1*Genes Expression during Post-Harvest Storage for Food Industry Application

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ABSTRACT - As one of world's biggest tropical fruits producer, Indonesian fruit production is not accompanied by high quality of fruit. Poor traditional post-harvest management practices usually leads to bad quality and wasted of fruit. In industrial scale, producing a consistent banana of high quality efficiently is crucial to reduce loss. In order to develop a good post-harvest technology, studies to create optimum environment condition, distribution, and storage are needed. The aims of this study are to study the effect of banana storage using temperature and fungicide treatment on ripening process, especially expression of *Musa* sp. cultivar pisang raja bulu*MaACS1* gene. The result showed that fungicide and storage at 20^0C treatment was the best storage condition for banana fruit and increasing banana shelf-life time. Semi-quantitative analysis on ripening-related gene, *MaACS1*, showed an increase of expression during ripening process with *MaACS1* gene expression at best storage condition was relatively lower compared to control.

Keywords: pisang raja bulu, MaACS1, gene expression, semi-quantitative PCR

FULL PAPER WITHDRAWED by AUTHOR

FOOD - 16

Expression Study of Pathogenic Resistance Genes for Genetic Improvement of Banana

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ABSTRACT - Banana is one of the world's most important trade commodities. However, infection of banana pathogenic fungi (*Fusarium oxysporum* race 4) is one of major causes of decreasing production in Indonesia. Genetic engineering has become an alternative way to control this problem by isolating genes which involved in plant defense mechanism against pathogens. Two of the important genes are *API5* and *ChiI1*, each gene encodes apoptosis inhibitory protein and chitiase enzymes. The purpose of this study was to study the expression of *API5* and *ChiI1* genes as candidate pathogenic resistance genes. The amplified fragments were then cloned, sequenced and confirmed with *in* silico studies. Based on sequence analysis, it is showed that partial *API5* gene has putative trans-activation domain and *ChiI1* has 9 chitinase family GH19 protein motifs. Data obtained from this study will contribute in banana genetic improvement.

Keywords : *Arabidopsis thaliana*, *Apoptosis inhibitor 5 (*API5), Chitinase (*ChiI1*), *Musa acuminata*, *Pathogen resistance*

FULL PAPER ACCEPTED by HAYATI JOURNAL

Somatic Embryogenesis from Male Flowers of Cavendish banana (*Musa acuminata* Colla, AAA)

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ABSTRACT - Cavendish banana is a triploid cultivar that is difficult to be improved genetically. Somatic embryogenesis has been known as a favorable propagation method. The objective of this study was to develop an optimal protocol for the establishment of local cultivar Cavendish banana using somatic embryogenesis method. Explants of male flowers between the twentieth and fifth bracts were induced to form yellow friable callus by supplementing *2,4*-dichlorophenoxyacetic acid (2,4-D), (1-naphthaleneacetic acid) NAA and indole acetic acid (IAA) into MS medium. For proliferation, thiadzuron (TDZ) was added to the medium. The cells were left until the somatic embryo reached globular stage. Embryogenic callus was the initial material for cell suspension cultures. Repeated sub-culturing and filtering every three weeks was done to maintain small aggregates and free floating single cells. This cell suspension was potential to be regenerated into plantlet. After four weeks, embryos were germinated into plantlet by shoot and root induction, after eight weeks on cultured medium.

Keywords: Cavendish banana, germination, male flowers, somatic embryo.

Addition of plant extracts on the production of coconut sugar and antioxidant activity evaluation

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ABSTRACT - Three kinds of coconut sugar including liquid, solidified and granulated form were usually produced by heating coconut sap material. Our research focused on how to improve quality of the products to be as a functional food candidate. Addition of plant extracts during coconut sugar production is one of our strategy to increase antioxidant activity of the products. The aim of the research was to determine the effect of plant extract addition on production of coconut sugar and to compare antioxidant activity among the products. Mangosteen rind, betel leaf, and clovers leaf were extracted with hot water (1:10 ml) at 70ºC for 20 minute, filtered through filter paper Whatman no 2. The supernatant in the concentration of 20, 40 and 60 ml / L of sap was added during heating of coconut sap, and the processes were continued until it concentrated, solidified and formed a granule. Antioxidant activities of the products were determined by FTC method. Result of the experiment indicated that increasing the amount of plant extract addition further increased antioxidant activity of coconut sugar. Antioxidant activities of the sugars subsequently from high to low were liquid > solidified > granulated coconut sugar. Furthermore, addition of mangosteen rind extract at 60 ml/L of coconut sap showed the highest antioxidant activity of the products.

Keywords: coconut sap; coconut sugar; plant extract; antioxidant activity.

Introduction

Coconut sugar is produced by heating coconut neera and further are made in three types; liquid, solid and granulated forms, respectively. Coconut sugar is commonly used in various types of traditional cuisine and beverages as a sweetener, flavor and color enhancer. Since the sugar has unique flavor, it become popular as a flavoring reagent for several foods. In addition, trend consumer on the consumption of natural foods has resulted in the use of coconut sugar as an natural alternative sweetener (Phaichamnan *et al.*, 2010). Coconut sugar also provides a sweet taste and low in calories, contains elements of proteins, mineral salts and rich in nutrients. Nutrient composition of coconut sugar per 100 g were 0.386 kcal of energy, 76 g carbohydrate, 10 g fat, 3 g protein, 76 mg calcium, phosphorus 37 mg, and 10 g of water (Said, 2007).

During coconut sugar production, Maillard reaction is occured and produces a brown color product called melanoidin (Winarno, 1997) that shows potential as an antioxidant (Zhuang and Sun, 2011), especially from high molecular weight (Dedin *et al.,* 2006). To enhance the functional value of coconut sugar, addition of antioxidant (natural antioxidants or synthetic antioxidants) during coconut sugar production is one of our strategies. The source of natural antioxidant including spices (ginger and turmeric), mangosteen rind, green betel leaf and clovers leaf. Mangosteen rind was rich antioxidant compounds such as anthocyanin, xanthones, tannins, and phenolic acids (Permana, 2010). In the other hand, betel leaves have antioxidant activity namely flavonoids, alkaloids, polyphenols, tannins and essential oils (Andarwulan *et al*., 1996) or eugenol component which some act as antioxidants to inhibit lipid peroxidation in clover leaf (Ogata *et al.,* 2000).

Materials and methods

a. Materials, Coconut neera, green betel, mangosteen rind, and clover leaf were obtained from local farmer in Banyumas regency, Central Java province. All chemical reagents were purchase from Sigma and Merck, except when stated in the text.

b. Preparation of betel leaf, clover leaf and mangosteen rind extract, Betel leaf, mangosteen rind, and clover leaf were dried on cabinet dryer at 60ºC and subsequently crushed into powder (60 mesh), extracted with water (1:10 w / w) and heated at 70°C for 20 minutes, filtered using a filter paper Whatman No. 2 to obtain the supernatant.

c. Production of liquid, solidified and granulated coconut sugar, Fresh coconut neera was filtered and heated up to ± 95°C, then the plant extract in the concentration of 20, 40 and 60 ml / L of neera were added. The process then continued to produce liquid, solidified and granulated coconut sugar.

d. Antioxidant activity, Antioxidant activity of sugar was determined by Ferric Thyocianate (FTC) and Thiobarbituric Acid (TBA) methods (Zahin *et al*., 2009). The FTC method was used to measure the amount of peroxide at the beginning of lipid peroxidation, in which peroxide react with ferrous chloride to form ferric ion. The ferric ion then combine with ammonium thiocyanate and produce ferric thiocyanate. The substance is red in colour. The TBA methods measures free radicals present after peroxide oxidation.

Ferric Thyocianate method: A mixture of 4.0 mg sugar sample in 4 ml absolute ethanol, 4.1 ml of 2.5% linolenic acid in absolute ethanol, 8.0 ml of 0.05M phosphate buffer (pH 7.0) and 3.9 ml of water was placed in a vial with a screw cap and then placed in an oven at 40 °C in the dark. To 0.1 ml of this solution was added 9.7 ml of 75% ethanol and 0.1 ml of 30% ammonium thiocyanate.

Precisely 3 minute after addition of 0.1 ml of 0.02M ferrous chloride in 3.5% HCl to the reaction mixture, the absorbance of red colour was measured at 500 nm each 24 hr until the day after absorbance of control reached maximum. BHT and α-tocopherol were used as positive controls while the mixture without sugar sample was used as the negative control.

Thiobarbituric acid method : Two ml of 20% trichloroacetic acid and 2 ml of 0.67% 2-thiobarbituric acid was added to 1 ml of sample solution, as prepared in FTC method. The mixture solution was heated in water bath and, after cooling, centrifuged at 3000 rpm for 20 min. Absorbance of the supernatant was measured at 552 nm. Antioxidant activity was determined based on the absorbance on the final day of FTC method.

e. Total phenol, Total phenol of sugar was determined by spectrophotometer (Shahidi & Naczk, 2004). To 0.50 ml of each sugar sample, 2.5 ml of 1/10 dilution of Folin-Ciocalteau's reagent and 2 ml of Na2CO3 (7.5%, w/v) were added and incubatedon at 45 °C for 15 min. The absorbance of all samples was measured at 765 nm using a Spectrophotometer. Milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g dw) was expressed as total phenol content in the sample.

f. Browning intensity, Browning intensity of the sugar was determined by spectrophotometer (Amin *et al*. 2010) with slight modifications. The sample solution prepared in dilution using distilled water (sample extract / distilled water) (1: 9,v/v). Absorbance of sample was measured at 420 nm using UV-Vis spectrophotometer (Shimadzu, model UV-2450).

Results and Discussion

Antioxidant activity

Antioxidant activity of coconut sugar was determined by ferric thyocianate method (FTC) based on the formation of peroxide which is the result of linoleic acid oxidation (Lestario *et al.*, 2005). This method was performed to measure the amount of peroxide formed and to determine the ability of antioxidants to inhibit the initiation of the reaction rate on the process of lipid oxidation. Peroxide absorbance value is inversely proportional to its antioxidant activity (Arinanti *et al.*, 2006). The antioxidant activities of liquid, solidified and granulated coconut sugar were presented in Figure 1.

Figure 1 showed the addition of clover leaf extract, mangosteen rind extract and combination clover leaf - mangosteen rind extract influence the antioxidant activity of the products and successively from high to low were liquid > solidified > granulated coconut sugar. The high antioxidant activity of liquid coconut sugar was correspond to low end point temperature during production of liquid sugar production (max. 100ºC) in comparison to solidified (105ºC) and granulated coconut sugar (120ºC). Previous studies reported that extracts of natural antioxidants were susceptible to heat damage (Anese *et al.*, 1999; Jeong *et al*., 2004; Azman Abdul Rahim *et al.*, 2010). Therefore intensified natural antioxidant extracts exposed to heat might increase the damage of antioxidant compounds.

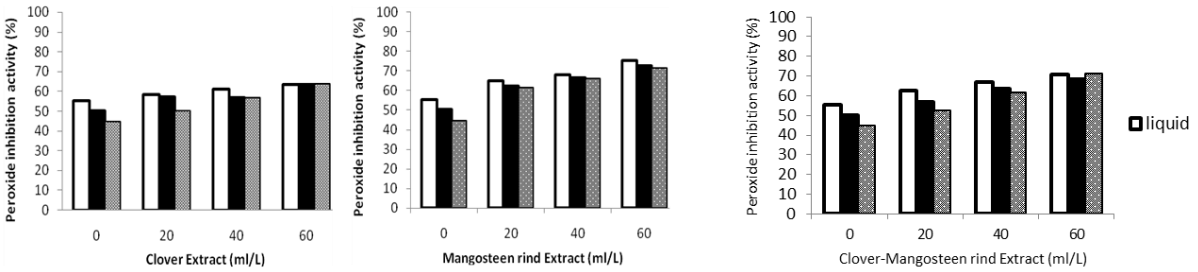

Figure 1 Peroxide inhibition activity of liquid, solidified and granulated coconut sugar in various concentration of clover leaf extract, mangosteen rind extract and combination of clover leaf-mangosteen rind extract. The data represents the mean of three independent experiments.

Addition of mangosteen rind extract showed the highest peroxide inhibition activity of sugar in a range of 61-75%, followed by addition of combination clover leaf- mangosteen rind extract with range 52-71%, and clover leaf extract which range from 50-64%. Mangosteen rind extract is known rich in antioxidants, especially anthocyanins, xanthones, tannins, and phenolic acids and showed antioxidant capacity of 84.6 to 86.29% (Permana, 2010).

Based on Figure 1, it also was observed that antioxidant activity was affected by the amount of plant extract addition. Increasing the amount of plant extract addition tend to increase the peroxide inhibition activity of sugar. The high antioxidant activity is related to the high content of total phenol. It was well knwon that phenol compounds was strong correlation with antioxidant activity (Xu & Chang, 2007). Phenol is the most important compounds that responsible to antioxidant activity found in plants (Caillet *et al.*, 2006). The mechanism of antioxidant activity of phenol compounds in the oxidation process was by providing H atoms that would bind to the peroxide product and produced a more stable compound (Gordon, 1990). Total phenol has primary mechanism of antioxidants by reacting with lipid radicals to produce high-energy product that has a better thermodynamic stability (Shahidi and Naczk, 2004).

Increasing concentration of plant extract addition tend to increase phenolic compounds accumulation in the product that imply to high antioxidant activity (Xu and Chang, 2007). The addition of clover leaf extract with a concentration of 800 ppm in beef and chicken sausage was better in inhibiting hydroperoxide formation compared with the concentration of 200 ppm (Syaiful, 2010).

Antioxidant activity of sugar was also determined by thiobarbituric acid (TBA) method base on formation of malonaldehide. Malonaldehide is one of the aldehide compounds produced from fat oxidation reaction. Malonaldehide values obtained by testing using TBA to determine the ability of antioxidants to inhibit the rate of termination reactions on lipid oxidation process. Malonaldehyde absorbance value inversely proportional to the antioxidant activity. The higher the mean absorbance value of the lower antioxidant activity, and the higher the number malonaldehyde the antioxidant activity was lower. Antioxidant activity of sugar base on TBA test were presented in the Figure 2.

Base on Figure 2, increasing plant extract addition tend to increase malonaldehide inhibition on the product. However, there were no significantly different on malonaldehyde inhibition among them.

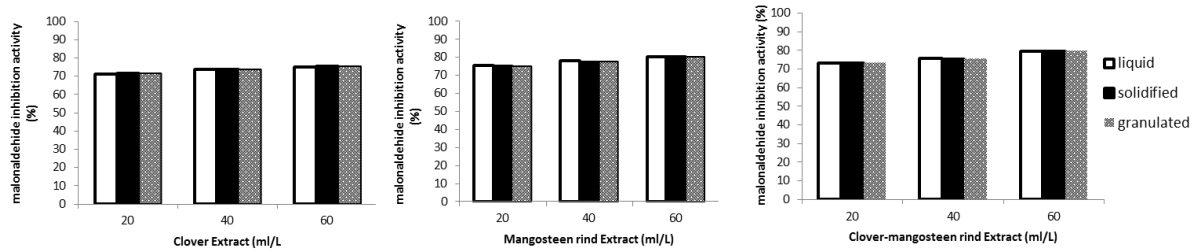

Figure 2 Malonealdehide inhibition activity of liquid, solidified and granulated coconut sugar in various concentration of clover leaf extracts, mangosteen rind extracts and combination of clover leaf-mangosteen rind extracts. The data represents the mean of three independent experiments.

Total phenol

Phenol is a compound with a hydroxyl group (OH), which can inhibit lipid oxidation by donating hydrogen atoms to free radicals (Fennema, 1996). Performing total phenol analysis is basic test for antioxidant activity, since it is well known that some phenolic compounds act as antioxidant to inhibit the oxidation process (Vermerris and Nicholson, 2006). A phenol compound was belong to primer antioxidant group (Kochhar and Rossel, 1990). The effect of addition of clover leaf extract, mangosteen rind extract and their combination on antioxidant activity of coconut sugar were presented in Figure 3.

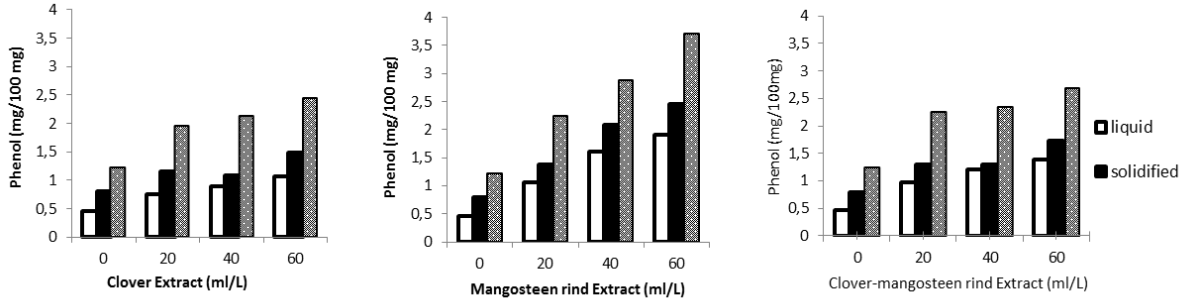

Figure 3 Phenol content of of liquid, solidified and granulated coconut sugar in various concentration of clover leaf extract, mangosteen rind extracts and combination of clover leaf-mangosteen rind extracts. The data represents the mean of three independent experiments.

The addition of Mangosteen rind extract resulted in the highest total phenol in coconut sugar ranged from 1.07 to 3.72 mg/100 mg, followed by the combination of clover leaf - mangosteen rind extract in a range between 0.98 to 2.69 mg/100 mg and clover leaf extract ranged from 0.75 to 2.45 mg/100 mg; respectively. The main antioxidants compound in mangosteen rind was xanthones which have high antioxidant activity than other antioxidant compound in plant materials (Hadriyono, 2011). The possibility of total phenol content in mangosteen rind extract was higher compared to the clovers leaf extract were confirm in this phenomenon. Clover leaf extract containing eugenol, and act as antioxidants by inhibiting fatty acid oxidation process that works almost the same as α-tocopherol (Ogata *et al.*, 2000). The high total phenol in combination with clover leaf extract - mangosteen rind showed a good synergistic effect between two types of antioxidant components of the extract. Figure 2 also showed that increasing addition of plant extract tend to increase total phenol content of sugar. The addition of the plant extract at 60 ml / L was gave higher total phenol content than the addition of 20 and 40 ml / L. This might be due to more phenol components accumulated in the product which was correspond to high amount of plant extract addition.

Browning intensity

Maillard reaction is non-enzymatic reactions between reducing sugars and amines, amino acids, peptides or proteins. It play an important role in formation of compounds responsible for unique flavours and colors in many kinds of foods. Browning intensity analysis was used to assess the extent of Maillard reaction occured in the sample, typically measured by absorbance at a wavelength of 420 nm (Amin *et al.,* 2010). Maillard reaction that occurred in production of coconut sugar produced melanoidin which had antioxidant activity, especially in high molecular weight (> 100 kDa) (Dedin *et al.*, 2006). Melanoidin has been demonstrated have antioxidant activity against oxygen radicals and chelating metal (Zhuang and Sun, 2011). Browning intensity of liquid, solidified and granulated of coconut sugar treated by addition of clover leaf extract, mangosteen rind extract and their combination were presented in Figure 4.

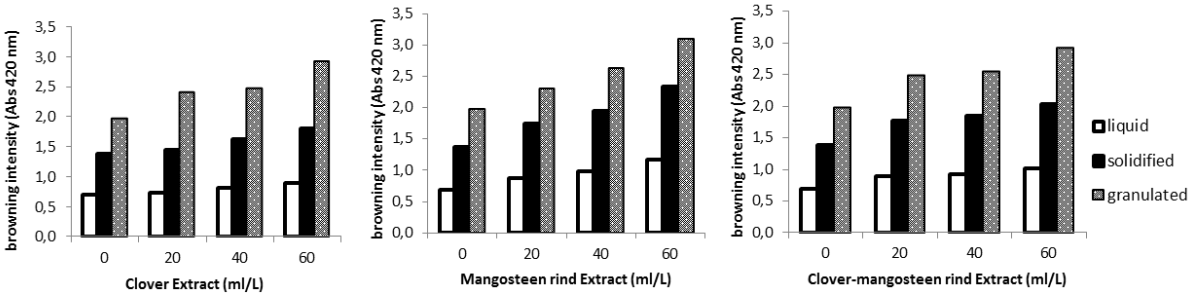

Figure 4 Browning intensity of liquid, solidified and granulated coconut sugar in various concentrations of clover leaf extracts, mangosteen rind extracts and combination of clover leaf-mangosteen rind extracts.The data represents the mean of three independent experiments.

Figure 4 showed that browning intensity values from high to low were liquid > solidified > granulated coconut sugar; respectively. Addition of mangosteen rind extract produced a browning intensity ranged from 0.87 to 3.09 mg/100 mg, followed by the combination of clover leaf extract - mangosteen rind with range between 0.89 to 2, 91 mg/100 mg, and the lowest browning intensity was in clover leaf extract ranged from 0.72 to 2.9 mg/100 mg.

The formation of brown color will increase with increasing pH of coconut neera, temperature and moisture content (Rosida, 2009). The pH value of clover extract, mangosteen rind extract and their combination were 4.5; 5.3; and 4.7; respectively. The high pH of coconut neera was imply to the color of sugar will be dark brown, due to Maillard reaction was intensively occurred (Catrien *et al.,* 2008). The pH of mangosteen rind extract was higher than the other extracts.

Figure 4, showed the relationship between the amount of plant extract and browning intensity. The amount of plant extract addition was directly proportional to browning intensity, meaning that high amount of extract addition, tend to high absorbance value of the products. The acid content in plant extract would cause increasing in the reduction of sugar which in turn will accelerate the browning reaction during heating of sugar (Windarwati, 2006). The hydrolysis of sucrose into reducing sugars was due to acid of the glycosidic bond leading to split water molecules, H+ ions from water attached to the OH- ions glucose and fructose and is attached to a reactive again (Maryani *et al*., 2010). The high concentration of acid was imply to more H+ ions was formed, so that the process of splitting sucrose into reducing sugar was also faster (Windarwati, 2006). Increasing reducing sugars concentration tend to increase browning reactions between amino acids and reducing sugars and the color of coconut sugar getting brown to dark (Rahayu *et al.* 2005).

Conclusion

Increasing the amount of plant extract addition further increases antioxidant activity of coconut sugar. Antioxidant activity of the sugar in various concentration addition of plant extract from high to low subsequently were liquid > solidified and > granulated coconut sugar. Furthermore, adition of mangosteen rind extract at 60 ml/L to coconut neera showed the highest antioxidant activity of the products. Further studies are needed to evaluate sensory characteristic and self life of the sugar.

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References

Amin, NAM., Mustapha, WAW., Maskat, MY., Wai, HC. Antioxidative Activities of Palm Sugar-like Flavouring. *The Open Food Science Journal* 23-29, 2010.

Andarwulan, N., Wijaya, CH., Cahyono, DT. Aktivitas Antioksidan dari Daun Sirih Hijau (*Piper betle* L.) [The antioxidant activity of green betel leaf]. Buletin Teknologi dan Industri Pangan VII(1): 29-37, 1996.

Anese, M, Manzocco, L., Nicoli, MC., Lerici, CR. Antioxidant Properties of Tomato Juice as Affected by Heating. *Journal of the Science of Food and Agriculture* 79:750-754, 1999.

Arinanti, M., Marsono, Y. Noor, Z. *Aktivitas Antioksidan pada Berbagai Jenis Kacang* [Antioxidant Activity in Various Types of Beans]. Jurnal Program Studi Ilmu dan Teknologi Pangan Sekolah Pasca Sarjana UGM. Fakultas Teknologi Pertanian Universitas Gajah Mada, Yogyakarta. 7p, 2006.

Azman Abdul Rahim, MS., Salihon, J., Yusoff, MM., Abu Bakar, I., Rizal Martua Damanik, M. Effect of Temperature and Time to the Antioxidant Activity in *Plecranthus amboinicus* Lour. *American Journal of Applied Sciences* 7(9): 1195-1199, 2010.

Caillet, S., Salmieri, S. Lacroix. M. Evaluation of Free Radical-Scavengering Properties of Commercial Grape Phenol Extracts by a Fast Colorimetric Method. *Journal of Food Chemistry* 95:1-6, 2006.

Catrien, YS. Surya, Ertanto, T. *Reaksi Maillard pada Produk Pangan* [Maillard Reaction in Food Products]. Program Kreativitas Mahasiswa Penulisan Ilmiah. Institut Pertanian Bogor, Bogor. 10p, 2008.

Dedin, FR., Fardiaz, D., Apriyantono, A. Andarwulan, N. Isolasi dan Karakterisasi Melanoidin Kecap Manis dan Peranannya Sebagai Antioksidan [Isolation and Characterization of Soy Sauce Melanoidin and Its Role as an Antioxidant]. *Jurnal Teknologi dan Industri Pangan* XVII(3): 204-213, 2006.

Fennema, OR. 1996. *Food Chemistry*. Springer-Verlag, Berlin.

Gordon, MH. *The Mechanism of Antioxidant Action iIn Vitro*. in Hudson, B. J. F. editor. Food Antioxidant. Elsevier Applied Science, London. 1-15, 1990.

Hadriyono, KRP. *Karakter Kulit Buah Manggis, Kadar Polifenol dan Potensi Antioksidan Kulit Buah Manggis (Garcinia mangostana L.) pada Berbagai Umur Buah dan Setelah Buah Dipanen* [Characteristics of Mangosteen Fruit Skin, Polyphenols Content and Antioxidant Potential of Mangosteen Fruit Skin (*Garcinia mangostana* L.) in various Age and After Fruit Harvested]. Skripsi. Fakultas Pertanian Institut Pertanian Bogor, Bogor.65hal. (On-line): http://repository.ipb.ac.id/bitstream/handle/123456789/48126/A11krp.pdf(acessed on 7 April 2013), 2011.

Jeong, S-M., Kim, S-Y., Kim, D-R., Jo, S-C. Nam, KC., Ahn, DU., Lee, S-C. Effect of Heat Treatment on the Antioxidant Activity of Extracts from Citrus Peels. Journal of Agricultural and Food Chemistry. 52: 3389-3393, 2004.

Kochhar, SP. & Rossel, JB. *Detection, Estimation and Evaluation of Antioxidant in Food Systems.* Pp. 19-64. In: B.J.F. Hudson (Ed). Food Antioxidant. Elservier Applied Science, London and New York. 317 p., 1990.

Lestario, LN., Hastuti, Rahardjo, Tranggono Sifat Antioksidatif Ekstrak Buah Duwet (*Syzygium cumini*) [Antioxidative properties of Duwet Fruit (*Syzygium cumini*) Extract]. Journal Agritech 25(1): 24-31, 2005.

Maryani.T., Surti, Ibrahim, R. Gelatin Application of Nile Tilapia (*Oreochromis niloticus*) Bone to the Quality of the Jelly Candy. *Jurnal Saintek Perikanan* 6(1): 62-70, 2010.

Ogata, M., Hoshim, M. Rano, S., Endo, T. Antioxidant Activity of Ezetimibe in Hyperchloresterolemic and Dimeric Compounds. *Chemical Pharmacy Buletin* 48: 147-149, 2000.

Permana, AW. *Peluang Tepung Kulit Buah Manggis sebagai Produk Minuman Instan Kaya Antioksidan* [The potency of mangosteen skin flour as instant beverage products with rich antioxidant] (On-Line). http://pascapanen.litbang.deptan.go.id. (accessed on 24 Februari 2013), 2010.

Phaichamnan M, Posri W, Meenune M. Quality Profile of Palm Sugar Concentrate Produced in Songkhla Province. *Thailand International Food Research Journal* 17: 425-432, 2010.

Rahayu, A., Suranto, Purwoko, T. Analisis Karbohidrat dan Lemak pada Pembuatan Kecap Lamtoro Gung (*Leucaena leucocephala*) terfermentasi *Aspergillus oryzae* [Analysis of Carbohydrates and Fats in the Processing of Soy Sauce Lamtoro Gung (*Leucaena leucocephala*) Fermented by *Aspergillus oryzae*]. Jurnal Bioteknologi 2(1): 14-20, Mei 2005, ISSN: 0216-6887, 2005.

Rosida, DF. *Aktivitas Antioksidan Fraksi-Fraksi Moromi, Kecap Manis dan Model Produk Reaksi Maillard Berdasarkan Berat Molekul* [Antioxidant activity of Moromi Factions, Sweet Soy Sauce and Maillard Reaction Products Model Based on Molecular Weight]. [Disertasi]. Bogor: Sekolah Pascasarjana, IPB. Bogor. (On-line) :http://repository.ipb.ac.id/bitstream/handle/123456789/22426/2009dfr.pdf?sequence=2. (acessed on 20 April 2013), 2009.

Said, A. *Coconut Sugar Production*. Ganesa Exact, Jakarta 52p., 2007.

Shahidi, F. & Naczk, M. *Phenolics in Food and Nutraceuticals*. CRC Press, New York. 566pp, 2004.

Syaiful, F. *Pengaruh Penambahan Ekstrak Etanol Cengkeh (Eugenia caryophyllata Thunb) dalam Sosis untuk Penghambatan Kerusakan Oksidatif Lemak.* [The Effect of Clove Ethanol Extract Addition on Sausage for Inhibition of Fat Oxidation]. [Skripsi]. Bogor: Program Studi Ilmu Pangan, Institut Pertanian Bogor.(On-line): http://repository.ipb.ac.id/bitstream/handle/123456789/41039/Bab%201%202010fsy.pdf. (acessed on 12 Mei 2013), 2010.

Vermerris, W. & Nicholson, R. *Phenolic Compound*. Netherlands: Springer. 88-90, 2006.

Winarno, FG. *Food Chemistry and Nutrition*. Gramedia, Jakarta. 253p, 1997.

Windarwati, S. *Kajian Pengaruh Hidrolisis Asam terhadap Karakteristik Gula Palma Cair* [Study on the Effect of Acid Hydrolysis on Palma Liquid Sugar Characteristics]. [Skripsi]. Fakultas Teknologi Pertanian, IPB. Bogor. (On-line): http://repository.ipb.ac.id/bitstream/handle/123456789/48423/A1krp.pdf. (acessed on 12 Mei 2013), 2006.

Xu, BJ., Chang, SKC. A Comparative Study on Phenolic Profiles and Antioxidant Activities of Legumes Affected by Extraction. *Journal of Food Science* 72(81): 59-66, 2007.

Zahin, M., Aqil, F., Ahmad, I. The In Vitro Antioxidant Activity and Total Phenolic Content of Four Indian Medical Plants. *International Journal of Pharmacy and Pharmaceutical Sciences* 1(1): 88-95, 2009.

Zhuang, Y, Sun, L. Antioxidant Activity of Maillard Reaction Products from Lysine-Glucose Model System as Related to Optical Property and Copper (II) Binding Ability. *African Journal of Biotechnology* 10(35): 6784-6793, 2011.

In vitro Regeneration of Foxtail Millet (*Setaria italica (L) Beauv*) cv. Buru hotong

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ABSTRACT - An experiment on *in vitro* regeneration of foxtail millet (*Setaria Italica* (L.) Beauv) has been done by using basal shoot explant of 10-days-old seedlings. Explants were cultured in MS basal medium supplemented with 2,4-D (0.1, 0.5, 0.6 ppm), kinetin (0.5, 1, 2 ppm), BAP (0.5, 1, 2 ppm) and 1.5 ppm $NiSO_4$ for shoot induction. Shoot multiplication and root induction were done in MS basal media without addition of plant growth regulator. Plantlets were then acclimatized in rice husk charcoal, cocopeat, or mixed media containing rice husk charcoal, humus and coarse fern powder (1: 2: 1). Results showed that MS basal medium containing 0.5 ppm kinetin, 2 ppm BAP, and 0.1 ppm 2,4-D was an optimal media for shoot induction, around 60% explant developed direct shoot organogenesis. The medium containing 1 ppm kinetin, 1 ppm BAP, and 0.5 ppm 2,4-D was an optimal medium for callus formation. Shoots were multiplied following transferred into MS basal media without growth regulator. Same condition occurred for root organogenesis, shoots developed burst of root after transferred into MS medium without growth regulators. Highest survival rate of plantlets (47%) were obtained when acclimatized in rice husk charcoal. Based on these results, it can be concluded that optimal media for direct shoot organogenesis was MS medium containing 0.5 ppm kinetin, 2 ppm BAP, and 1 ppm 2,4-D. The best survival rate for acclimatization was gained in rice husk charcoal media.

Keywords: Acclimatization, BAP, dichrolophenoxyacetic acid (2,4-D), foxtail millet (*Setaria italica (L.) Beauv*), kinetin, MS basal media, organogenesis, rice husk charcoal.

FULL PAPER ACCEPTED by ITB JOURNAL

The bioprospect of *Croton tiglium* L. and *Ricinus communis* L. as a source material for biodiesel

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ABSTRACT - Plant members of the Euphorbiaceae family, such as *Jatropha curcas* (physic nut) and *Ricinus communis* (castor oil), are ~~a~~ well known biodiesel source. This research aimed to compare the prospect of another species of Euphorbiaceae's family, *Croton tiglium,* with *Ricinus communis* as biodiesel source. Those plants were sampled from (BALITRO), Bogor. Potency of seed oil production was determined by estimating seed productivity per hectare; identification of fatty acids composition using GCMS (Gas Cromatography Gas Spectrophotometer); measurement of seed oil quality based on saponification number, as well as iodine value. Seed oil produced by both species were almost equal, *C. tiglium* produced 0,51 ton/ha, while *R. communis* produced 0.47 ton/ha. content of seed oil produced by *R. communis* (58.5%) was richer than *C. tiglium* (53,99%). The component of methyl ester oil *C. tiglium* were saturated, monounsaturated, and polyunsaturated were 23.76 %, 27.66%, 47.761 % respectively whereas literature study of *R. commnunis* oil content showed respectively 91.8 %, 3.7%, 4.8 %. Quality of seed oil produced by both species, based on saponification number and iodine value, are fulfilled SNI no SNI-04-7182-2006. In conclusion, *R. commnunis* has more potential than *C. tiglium* to cultivated as biofuel source.

Keywords: biodiesel, *Croton tiglium,* oil content, productivity, *Ricinus communis*, saturated oil

Introduction

In the last few years, the potency of *Jatropha curcas* L. *and Ricinus communis* L.(Euphorbiaceae) had been studied by many researchers for the production of biofuel and industrial products.

This study was aimed to explore other species of Euphorbiaceae that have been utilized traditionally for many purposes, including medicine. Previous studies (Irwanto *et. al.*, 2007) using six species from different families, i.e *Terminalia catappa* Linn (Combretaceae), *Azadirachta indica* A. Juss (Meliaceae), *Tamarindus indica* Linn (Caesalpiniaceae), *Croton tiglium* (Euphorbiaceae), *Cerbera odollam* Gaertn. (Apocynaceae) and *Brassica rapa* (Brassicaceae) have showed that *Terminalia catappa* Linn (Combretaceae), *Azadirachta indica* A. Juss (Meliaceae), C*roton tiglium* (Euphorbiaceae), *Cerbera odollam* Gaertn. (Apocynaceae) have more than 30% oil. *Croton tiglium (*Kemalakian or Ceraken, Ind) also has a high oil content of 48%, which means a similar content to *Jatropha* (Mulyani, 2007) while *R. communis* has (40-50%) (Poteet, 2006). However, to be used as ~~a~~ biofuel source, seeds oil produced must meet requirements referring to SNI-04-7182-2006. Therefore, the aim of this research was to compare the potency of seeds oil produced by *C. tiglium* and *R. communis* as new source of biofuel.

Methods

The productivity of seed oil was determined by calculate percentage of oil content with total number of plants per hectare. The total number of plants was estimated by proportion of area covered by plant crown.

The seeds were collected from Ballitro, Bogor. Seeds were separated from fruit, dried at 25^{o}C for a few days, and then cut into pieces of 1- 2 mm. The seeds were then extracted on Soxhlet apparatus for 5 hours to obtain seed oil. Oil extract then analyzed for content.

The fatty acids composition was analyzed using GCMS (*Gas Cromatography Mass Spectrophotometer*) at Chemical Analysis Services Unit, LIPI. Oil quality was measured by saponification and iodine test. Saponification number was measured by titrimetric method FBI-A03-03 with alcoholic KOH as a solvent and HCl 0,5N as a standard titran. Iodine value was measured by Hanus method, with standard sodium tiosulfat 0,1N as a titran and Hanus reagent and phenolftalein as indicator.

Results and Discussion

Seed oil content

Oil content is defined as percentage of oil contained in a seed. The results show that *C. tiglium* has oil content of (53.99%), which is lower than *R. communis* (58.5%) (Figure 1). Oil content of *C. tiglium* used in this study was higher than (Azam *et. al.*, 2005) who mentioned that seeds oil content of *C. tiglium* was 45%. However, this finding is still in the range of values of 50-60%, as mentioned by Soerawidjaja (2006). The seeds oil content of *R. communis* within range published by the CSIR (Council for Scientific and Industrial Research) (31-61%) and higher than result published by (Scholz *et. al.*, 2008) (40-55%).

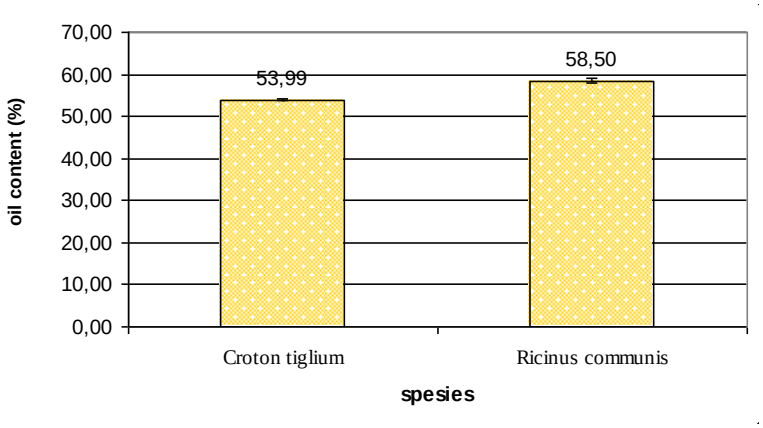

Figure 1 Comparison of oil content between *C. tiglium* and *R. communis*

Seed oil production

It is assumed that a hectare of land could supported 2500 *C. tiglium* trees and 1111 *R. communis* trees. However, each *R. communis* tree produces more fruits (1554) than *C. tiglium* (747) (Table 1). This is could be caused by differences in flowering type between both species as *R. communis* has huge inflorescences compare to *C. tiglium*. Even though produced more fruit per tree, productivity of fruit per ha of *C. tiglium* (1.87 $x10^6$) higher than *R. communis* (1.73 $x10^6$). Therefore, based on fruit production per ha, *C. tiglium* has more potency than *R. communis* (Table 1).

Table 1 Estimation of the total number of tree and fruit of *Croton tiglium* and *Ricinus communis*

Plant productivity	*Croton tiglium*	*Ricinus communis*
-total number tree/ha	2500	1111
-total number fruit/tree	747	1554
-total number fruit/ha ($x10^6$)	1.87	1.73

Table 2 Estimation of production of fruit, seeds and oil in *Croton tiglium* and *Ricinus communis*

Parameter	*Croton tiglium*	*Ricinus communis*
- number of fruit/ ha	1.87 $x10^6$	1.73 $x10^6$
- proportion of seeds to fruit	0.67	0.615
- fruit production (ton/ha)	1.78	1.795*)
- seeds production (ton/ha)	1.12	1.105
- oil production (ton/ha)	0.51	0.47

The proportion of seeds to fruit of *C. tiglium* (0.67) has a higher proportion than *R. communis*, which has a value of 0.61. These results indicate that *C. tiglium* has more potency to produce oil than *R. communis*, based on the proportion of seeds to fruit.

Table 3 shows a comparison of the productivity of the fruit and kernels and oil production between species *C. tiglium* and *R. communis*. The result shows that productivity of fruit per hectare of *R. communis* (1.795) is higher than in *C. tiglium* (1.78), although there is not much difference. Nevertheless, *C. tiglium* produce seeds, kernel and oil (1.12, 0.94, 0.51 tonnes per hectare) which were higher than *R. communis*, although the difference is also a slight number. This is because the proportion of seeds for fruit and kernels of the fruit of *C. tiglium* is greater than *R. communis*.

Table 3 A comparison of fruit, kernel and oil proctivity between *C. tiglium* and *R. communis*

Productivition	*Croton tiglium* (ton/ha)	*Ricinus communis* (ton/ha)
Fruit	1.78	1.75
Seed	1.12	1.105
Kernel	0.94	0.81
Seed oil	0.51	0.47

Estimation of seed production of *R. communis* from this study was similar to the statistics of FAO in 2005, with average seed production of *R. communis* per hectare was 1.1 tonnes. On the other hand, seed productivity of *C. tiglium* based on Duke (1983) was 0.9 tonnes/ha.

In comparison with other potential biodiesel crops, oil productivity of *R. communis* and *C. tiglium* is higher than cotton (0.12), ground nut (0.22), soybean (0.38), and sunflower (0.44). However, oil productivity was quite low compared to palm oil (*Elaeis guineensis*), (3.57) (Mielke, 2007).

Table 4 Saponification number and iodine value of of *Ricinus communis* and *Croton tiglium* in comparison to Indonesian Biodiesel Standard (SNI 04-7182-2006)

Biodiesel parameter	*Croton tiglium*	*Ricinus communis*	Indonesian Biodiesel Standard (SNI 04-7182-2006)
Saponification number (mg KOH/g biodiesel)	155.4	173.32	-
Iodine value (mg I2/100g biodiesel)	93.71	83.45	maximum 115

Table 4 showed that the *R.communis* saponification number (173.32 mg KOH/g) was higher than *C. tiglium*'s (155.4 mg KOH/g). However, higher saponification number indicated, lower oil mass molecule (Knothe, 2002). Then it could be predicted that *R. communis* has a fatty acid with a carbon chain length and molecular weight greater than *C. tiglum* (Ogunniyi, 2006). showed the saponification numbers of *R. communis* was 179-185 mg KOH/g of oil, which means that it was not that different compared to our results. Saponification numbers of *C. tiglium* in our study was low in comparison to the data collected by (Poteet, 2006), who obtained a saponification numbers of 203.9.

Iodine value is determined by the unsaturated quantity of biodiesel (Environment Australia, 2003). The iodine value of oil produced by *C. tiglium* (93.71 g-I_2/ 100g) was higher than *R. communis* (83.45 g-I_2 /100 g). According to SNI 04-7182-2006, maximum iodine value which is allowed for biodiesel source is 115 g-I_2 /100 g. Therefore, both seeds oil *of C.tiglium* and *R.communis* fulfilled its criteria as biodiesel sources. High iodine value indicated high unsaturated degree of biodiesel. High unsaturated degree of biodiesel could produce precipitation in machines which produce detrimental effects in long term (Knothe, 2002).

Fatty acid composition

Composition of fatty acids methyl esters of *R. communis* and *C. tiglium* oil could be grouped as saturated fatty acid and unsaturated fatty acid. The unsaturated fatty acid in *Croton tiglium* was higher than saturated fatty acid (Table 5).

Table 5 The composition of fatty acid methyl in *Croton tiglium* and *Ricinus communis*

Solution	*Croton tiglium*	*Ricinus communis*[a)]
Metil Ester saturated	23.76 %	91.8 %
Metil Ester monounsaturated	27.66 % (metil oleat 17.46%)	3.7 % (metil oleat 3%)
Metil Ester polyunsaturated	47.61 %	4.5 %

Source: www.castoroil.in and Ogunniyi (2005)

Ricinus communis has a higher saturated methyl ester (91.8%) than *C. tiglium* (23.76%). According to Demirbas (2008) content of saturated fatty acids and carbon chain length are proportional to the cetane number. Therefore, higher saturated fatty acids and longer carbon chain, will produce higher cetane make it environment friendly (Van Gerpen, 2005).

Ricinus communis has a high saturated methyl esters dominated by methyl ricinoleat, (89.5%) or acid 12-hydroxy-9-oktadekenoat. The presence of hydroxyl groups and double bonds makes these oils suitable for a variety of chemical reactions, however, hydrogen bonding of hydroxyl group also increase viscosity of seeds oil of *R. communis*. Ogunniyi (2006) mentioned that kinematic viscosity of seed oil of *R. communis* at 40°C was of 240-300 mm2s-1. This number was very high compared to the standard of ISO-04-7182-2006, which mentions the ideal of kinematic viscosity is in the range of 2.3 to 6.0 mm2s-1.

Oil produced from *C. tiglium* seeds contain 27.66% monounsaturated methyl ester (methyl oleate = 17.46%), while *R. communis* contain 3.7% monounsaturated methyl esters (methyl oleate = 3%). There is a positive relationship between oleic acid with cetane number (Bringe, 2005) the more oleatnya acid content, the higher cetane number the biodesel has.

Oil produced from *C. tiglium* seeds contain 47.671% polyunsaturated methyl esters, while *R. communis* has 4.8% polyunsaturated methyl ester. Compared with saturated fatty acids, polyunsaturated fatty acid easier to oxidized, but has a low cetane number and has difficulty forming new fuel compositions (Bringe, 2005) which will cause it to interfere with good quality oil.

Conclusion

Croton tiglium produces more seed oil than *Ricinus communis*. However, fatty acid methyl ester of oil of *Ricinus communis* was more suitable to be used as a biodiesel, and it also meets the specification of biodiesel standard in Indonesia.

Acknowledgements

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References

Azam, MM., Waris, A., Nahar, NM. Prospects and potential of fatty acid methyl esters of some non-traditional seed oils for use as biodiesel in India. *Biomass and Energy* 29:293-302, 2005.

Bringe, N.A. *Soybean Oil Composition for Biodiesel*. The Biodiesel Handbook Chapter 6.7: 159. AOCS Press, 2005.

Demirbas, A. Biodiesel: *A Realistic Fuel Alternative for Diesel Engines*. Springer-Verlag London Ltd., 2008.

Irwanto, R.R., Rofianty, R., Rahman, HA, Mulyani, E., Wardini, TH. *Bioprospecting of potential plants for bioenergy resources*. Proceedings of International Symposium "Food Security, Agricultural Development and Environmental Conservation in Southeast and East Asia". Bogor, Indonesia, 2007.

Knothe, G. *Structure Indices in FA Chemistry. How Relevant Is the Iodine Value?*. JAOCS, 79: 847–854 2002

Mielke, T. *Major Global Trends-Impacts from Biofuels Supply and Demand Outlook*. ISTA Mielke GmbH, Oil World, Germany. Presented at "Agrotendencias, Buenos Aires, May 29, 2007.

Mulyani, E., *Bioprospek Cerbera odollam Gaertn*. Taken from Three Locations For Biodiesel Raw Materials. Undergraduate Thesis, Biology Program, Institute of Technology Bandung, 2007.

Ogunniyi, D.S. Review Paper Castor Oil: A Vital Industrial Raw Material. *Bioresource Technology* 97:1086-1091, 2006.

Poteet, M.D. *Biodiesel Crop Implementation in Hawaii*. Hawaii Agriculture Research Center. Aiea, 2006.

Scholz & Scholz V and da Silva. Prospects and Risks of the Use of Castor Oil as a Fuel. *Biomass and Bioenergy* 32: 95-100, 2008.

Soerawidjaja, T.H. *Foundations-foundations Scientific and Engineering and Technology Biodiesel*. Presented at the National Seminar on "Biodiesel as an Alternative Energy Future". UGM Yogyakarta, 15 April 2006.

Van Gerpen, J. *The Basics of Diesel Engines and Diesel Fuels*. The Biodiesel Handbook Chapter 3. AOCS Press, 2005

Sustainability of the Palm Oil Industry through Oil Recovery and Creation of Products from Oil Palm Wastes

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ABSTRACT - In Malaysia, palm oil industry is the fourth largest contributor to the economy and the government has targeted to increase to RM178 billion by the year 2020. The government through PEMANDU has recognized initiative to increase the national OER as one of the important project in the Palm Oil NKEA. Historically, the average OER performance in the country has not made significant improvement and one of the factors is due to oil loss in the waste materials. Normally the residual oil in the EFB, DC and POME is not recovered because there is no proven technology and the fundamental mechanism of oil-water-fiber system is not well understood. Statistically, nearly 70% of FFB processed will end–up as waste materials such as MF, PKS, DC and EFB. Together with these, huge amount of POME is also produced. Many scientific papers have reported the available oil in these wastes, however no proper study has been conducted to recover it. The future of palm oil industry depends on the sustainability issues of the people, planet and profit. Thus the main objective of this paper is to highlight the economic value of oil in the oil palm wastes towards achieving zero waste strategy for the sustainability of the palm oil industry.

Keywords: Crude palm oil, decanter cake, empty fruit bunch, palm oil mill wastes, oil recovery, palm oil mill effluent.

Introduction

In Malaysia, the palm oil industry is currently at the fourth place in terms of gross national income (GNI) contribution to the country and by the year 2020, the Performance Management and Delivery Unit (PEMANDU) of Malaysia has targeted the industry will contribute RM178 billions of additional income and 42,000 new employment opportunities to the country (PEMANDU, 2012). Through Economic Transformation Programme (ETP) which was launched by the Prime Minister of Malaysia, the government has outlined four (4) main segments to be focused in the palm oil industry; 1. Plantation, 2. Palm oil milling and trading, 3. Non-food downstream and food and 4. Health based downstream. In order to achieve the vision, the government has identified strategic thrusts for sustainability in both upstream and downstream activities in the industry. One of the entry point projects (EPP) identified under the thrusts is to improve the crude palm oil (CPO) oil extraction rate (OER) in the palm oil milling process. It is estimated that for every 1% increase of OER, the country will benefit extra USD330 million (currency exchange rate USD1=RM3.3) each year from the palm oil industry. For the mill, for every 1% increase in OER, a mill will gain extra USD3.3 millions per year. The monetary impact of improving OER is huge and one of the promising focus area to increase OER is through oil extraction from the oil palm biomass materials such as oil palm empty fruit bunches (EFB), oil palm decanter cake (OPDC) and palm oil mill effluent (POME).

OER is a universal indicator to measure the actual amount of oil obtained from the fresh fruit bunch (FFB) pressed (Chang, 2003). The maximum OER from a ripe FFB is estimated to be 30%. Over the past few decades the OER has not made significant improvement and the reasons always been associated with soil, climate, oil palm tree varieties, poor milling operation and machinery efficiency [2]. Through literatures search, there are not much focus has been given to determine the oil loss in the palm oil wastes and wastewater produced and estimate its economic impacts. Therefore the objective of this paper is to give some insights on the value of CPO in the EFB, OPDC and POME and develop promising strategies to recover it.

Palm Oil Wastes

The types of palm oil wastes in the mill commonly known as oil palm biomass are empty fruit bunch (EFB), mesocarp fiber (MS), oil palm decanter cake (OPDC) and palm oil mill effluent (POME). These biomass share the same source i.e oil palm fresh fruit bunches (FFB) and classified as lignocellulosic material. Due to its amphiphilic properties, these biomass are prone towards absorbing high amount of oil within its fiber matrix and therefore difficult to be completely removed through normal physical methods. For example, the popular technology to recover oil from EFB is using mechanical pressing technique and solvent extraction using hexane. However, some of the issues such as efficiency, quality, safety and environmental issues could not be properly addressed using these techniques. Therefore there is an urgent need to propose a new and appropriate technique to enhance the oil recovery from oil palm wastes using the resources available in the mill area with green technology features and concepts.

In addition to solid wastes (EFB and OPDC), palm oil mill also produces a huge amount of wastewater known as POME. At present there is a growing trend of utilizing POME to produce biogas through anaerobic digestion technology (Sulaiman *et al.* 2009). Through observation and reported cases in many studies, in addition to high Biological Oxygen Demand (BOD), minerals and solids, POME also contains a small quantity of residual oil. Through daily observation in the palm oil mill at the sludge-pit area, there was a significant amount of oil floated on the surface of cooling and mixing ponds. It was clear that the efficiency of the sludge-pit required improvement because it was not designed to handle variation of the incoming POME characteristics such as high and low flowrates, solid contents, oil contents and temperatures fluctuation. Furthermore, poor sludge-pit maintenance

could also reduced its performance and efficiency due to solid accumulation at the bottom. Therefore it is important to further study to recover the remaining oil in POME to gain extra income for the mill and therefore improve the overall POME treatment system thereafter.

Crude Palm Oil Recovery Strategies

The palm oil wastes and wastewater generated in the palm oil mill still contains a significant amount of residual CPO and if the recovery process is fast, the quality of recovered oil can be maintained.

Table 1 Strategies that can be adopted to recover the residual oil in EFB, OPDC and POME.

Description	Strategies	Potential CPO Value
Recovery of CPO from EFB to improve the overall oil extraction rate (OER).	• Understanding the fundamental of fiber-oil surface tension and absorption science. • Improved design a new press-shredder machine.	660 tonnes CPO/mill/year. Assumptions: Processing capacity 1000 tonnes/day, operation 300 days, Oil content 2% in EFB, EFB is 22% of FFB, the recovery rate is 50%.
Recovery of CPO from OPDC.	• Fundamental understanding of the oil recovery from DC study. • Design a new system to recover oil from DC.	284 tonnes CPO/mill/year. Assumptions: Processing capacity 1000 tonnes per day, operation 300 days, oil content 4.5% in DC, DC is 42 kg/tonne FFB, CPO price is RM3000 per tonne, recovery rate is 50% .
Recovery of CPO from POM E.	• Understand the current problems with the existing sludge-pit design. • Propose a new and improved design system to recover CPO from POME.	700 tonnes CPO/mill/year. Assumptions: Processing capacity 1000 tonnes per day, operation 300 days, oil content 0.75% in POME, POME is 0.62 tonne per tonne of FFB, CPO price is RM3000 per tonne, the recovery rate is 50% .

Table 1 also shows the potential amount of CPO available in EFB, OPDC and POME. If the recovery technology is economically viable and able to maintain its quality, then it is justified to recover the residual oil. Table 1 also shortlists some of the strategies that can be adopted to recover the residual oil.

Sustainable Use of Palm Oil Wastes

Sustainability is always associated with the protection of the environment, social benefits and wellbeing and business profitability. To ensure the sustainability of the palm oil industry in the future, various efforts should be geared towards the sustainable development thrusts. In the palm oil mill,

three (3) types of major waste materials or biomass are produced. Those are EFB, OPDC and POME. The flowchart of their origins is presented in Figure 1.

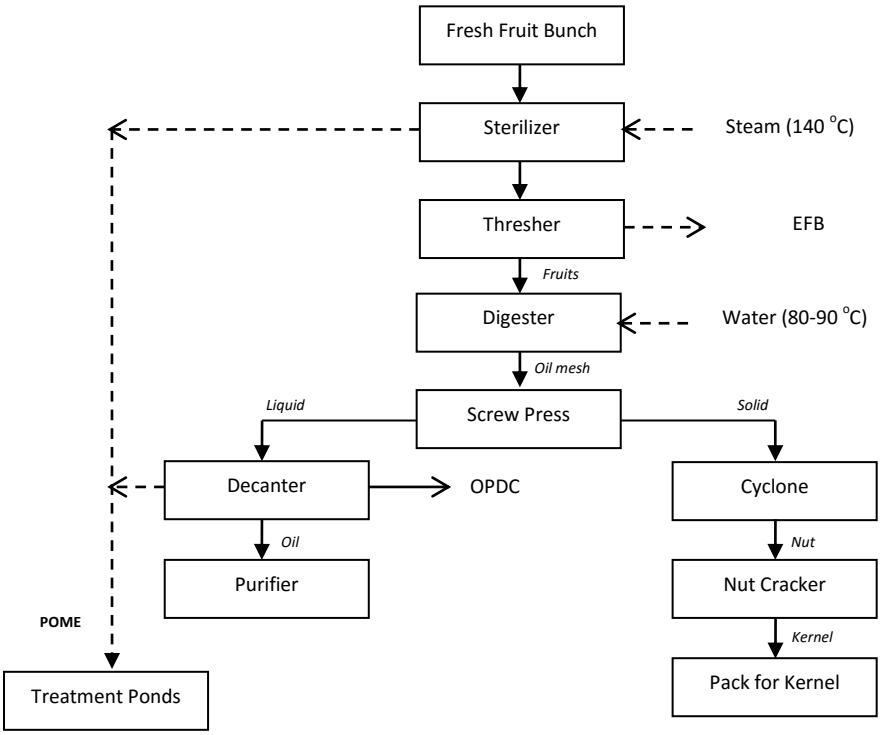

Figure 1 The waste materials production stage in the palm oil mill (Prasertsan & Prasertsan, 1996).

In the case of palm oil industry, the processing wastes known as oil palm biomass, must therefore be used as feedstocks to produce higher value added products. Therefore this section will review some of the common uses of oil palm biomass.

Oil Palm Empty Fruit Bunches

Oil palm empty fruit bunches (EFB) are obtained once majority of the loose fruits have been detached from the bunches through a process called threshing. The traditional method of using EFB is for mulching in the oil palm plantation. Mulching process helps the available nutrients in the EFB could be recycled into the soils.

Current understanding show that EFB could also be processed and turned into biocompost (Baharuddin *et al.* 2009; Baharuddin *et al.* 2010; Baharuddin *et al.* 2011). The biocomposting technology of EFB is currently used only for the in-house application rather than for commercial purpose. Through more advanced technology, studies reported that the fiber of EFB can be used to produce polyethylene biocomposite material (Mohd. Ishak *et al*. 1998; Rozman *et al.* 1998), polyester biocomposite material (Abdul Khalil *et al.* 2007), activated carbon (Tan *et al*. 2009), bio-oil (Abdullah *et al.* 2011) and bioethanol. Serious efforts have been geared towards its commercialization stage. Therefore the future use of EFB for higher value-added products could be a promising research, development and commercialization initiatives.

Oil Palm Decanter Cake

In the palm oil mill, oil palm decanter cake (OPDC) is produced at the clarification stage where fine solids are removed from the oil rich phase using a decanter machine (Gutiérrez *et al.* 2009).

Physically the colour it black, slightly acidic, high moisture content, contains a significant amount of cellulose and lignin, high amount of BOD and COD, silica and minerals.
It is currently used as a co-substrate for producing biofertilizer and animal feed. Due to its unpleaseant smells, some of the mills dispose-off the OPDC in the dumping ponds to degrade it naturally. To date there is no proper studies have been conducted to make use of OPDC material except for supplementation for biocomposting process (Yahya *et al.* 2010). Table 2 shows the typical characteristics of the OPDC produced in the mill as reported by different researchers (Paepatung *et al.* 2009; Yahya *et al.* 2010; Abdul Razak *et al.* 2012). By looking at its chemical characteristics, the future potential use of OPDC is very promising for the biofuel and biomaterial industry. The oil content in OPDC is 4.6%. Therefore it can be directly transesterified to produce biodiesel (methyl ester). It also contains a high percentage of cellulose and lignin which can be used as filler for plastic biocomposite materials.

Table 2 Chemical compositions of the DC produced in the mill

Parameters	**(Abdul Razak *et al.*2012)**	**(Yahya *et al.* 2010)**	**(Paepatung *et al.* 2009)**
Ph	4.08 ± 0.02	-	-
Moisture (%)	76.46 ± 0.8	76.38	76.70
VS (%)	-	-	83.40
Cellulose	21.61	-	
Hemicellulose (%)	3.94	-	
Lignin (%)	30.66	-	
Ash (%)	22.25	-	
COD (mg/kg)	316,937	-	880,000
TOC (mg/kg)	-	-	470,000
BOD (mg/kg)	41,813	-	
Oil and Grease (mg/kg)	43,000	-	46,200
TS (mg/kg)	582,000	-	
N (%)	2.80	2.38	
C (%)	-	-	43.60
C/N Ratio	19.70	51.70	
H (%)	-	-	5.79
O (%)	-	-	31.70
S (%)	0.30	0.39	0.15
P (%)	0.20	-	-
K (%)	1.40	2.39	-
Ca (%)	0.90	1.02	-
Mg (mg/kg)	0.30	0.80	-
Bo (mg/kg)	9.00	-	-
Mn (mg/kg)	38.00	-	-
Cu (mg/kg)	59.00	-	-
Fe (mg/kg)	4,438	-	-
Zn (mg/kg)	30.00	-	-
TKN (%)	-	-	21.50
NH_3-N	-	-	0.69

Palm Oil Mill Effluent

Palm oil mill effluent or in short known as POME is an organic rich wastewater that is produced at the end of palm oil milling process. The effluent is contributed by wastewater from three (3) main sources namely sterilization, clarification and hydrocyclone/claybath processes (DOE, 1999). The other minor sources of POME include press machine, engine room, boiler, strainer, desander, purifier, vacuum dryer and oil trap (DOE, 1999). The typical characteristics of POME in terms of pollution load are given in Table 3 as reported by several researchers (DOE, 1999; Najafpour *et al.* 2006). The

wastewater contains high a amount of BOD, COD and dissolved solids. It is slightly acidic with small percentage of oil. In comparison, the clarification source of POME contains highest amount of oil, followed by sterilization and lowest is hydrocylone/claybath source.

Table 3 Characteristics of palm oil mill effluent from sterilization, oil clarification and hydro-cyclone processes.

Parameters (values are in mg/L) except pH	**Sterilization**	**Oil clarification**	**Hydrocyclone**
Ph	5.0	4.5	-
Oil & Grease	4,000	7,000	300
Biochemical Oxygen Demand	23,000	29,000	5,000
Chemical Oxygen Demand	47,000	64,000	15,000
Suspended Solids	5,000	23,000	7,000
Dissolved Solids	34,000	22,000	100
Ammoniacal Nitrogen	20	40	-
Total Nitrogen	500	1,200	100

POME is a colloidal suspension with temperature from 80 - 90 ^{o}C, pH value 3.8-4.5, water 95-96%, 0.6-0.7% oil and grease, 4-5% total solids with appreciable amounts of plant nutrients (Ahmad *et al.* 2006; Najafpour *et al*. 2006; Zinatizadeh *et al*. 2006). In addition, POME was also reported to contain phosphorous (180 mg/L), potassium (2,270 mg/L), magnesium (615 mg/L), calcium (440 mg/L), boron (7.6 mg/L), iron (47 mg/L), manganese (2.0 mg/L), copper (0.9 mg/L), zinc (2.3 mg/L), nitrogen (950 mg/L), phosphorous (150 mg/L) and magnesium (345 mg/L) (Ahmad *et al*. 2006). The caloric value of POME was reported to be 16,992 kJ/kg (Foo & Hameed, 2009). Table 4 lists some of the nutrients that can be extracted from POME as reported in the literature (Wu *et al*. 2009).

Table 4 Major constituents, amino acids, fatty acids and minerals in POME.

Constituents	**%**	**Minerals**	**µg/g dry weight**
Protein (crude)	12.75	Fe	11.08
Lipid (crude)	10.21	Zn	17.58
Carbohydrate	29.55	P	14377.38
Nitrogen-free	26.39	Na	94.57
extract		Mg	911.95
		Mn	38.81
Amino acids	**%**	K	8951.55
Aspartic	9.66	Ca	1650.09
Glutamic	10.88	Cu	10.76
Serine	6.86	S	13.32
Glycine	9.43	Se	12.32
Alanine	7.70	Si	10.50
Methionine	6.88	Al	16.60
Fatty acids	**%**		
Myristic	12.66		
Palmitic	22.45		
Stearic	10.41		
Oleic	14.54		
Linoleic	9.53		

It is interesting that, despite of its high pollution load, POME also contains high nutrients such as carbohydrate, protein, lipid, carotene, amino acids, fatty acids and minerals as reported by previous research [22] which suitable for various purposes. It was also reported that because of these nutrients, some of the bioconversion process such as anaerobic digestion and biocomposting are favorable as a result of natural micronutrients supplementation to the available microorganisms. It is expected that the future trend of POME utilization would be focused on oil and nutrient recovery for higher value-added products.

Conclusion

In conclusion, the potential economic return to the industry from the oil recovery and the creation of value-added products is huge. It is expected that the palm oil industry can have additional income from the oil recovery projects from oil palm wastes. Through this project, the environmental protection and social well being of the surrounding communities can also be improved. Thus it is a timely opportunity for the palm oil industry to start considering the commercialization of oil recovery and creation of high value products for the sustainability of the palm oil industry.

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References

Abdul Khalil, H.P.S., _Hanida, S., Kang, C.W., Nik Fuaad, N.A. Agro-hybrid composite: The effects on mechanical and physical properties of oil palm fiber (EFB)/glass hybrid reinforced polyester composites, Journal of Reinforced Plastics and Composites 26(2):203-218, 2007.

Abdul Razak, M.N., Ibrahim, M.F., Phang, L.Y., Hassan, M.A., Abd-Aziz, S. Utilization of oil palm decanter cake for cellulase and polyoses production. *Biotechnology and Bioprocess Engineering* 17: 547-555, 2012.

Abdullah N., Sulaiman, F., Gerhauser, H. Characterisation of oil palm empty fruit bunches for fuel application,.*Journal of Physical Science* 22(1): 1–24, 2011.

Ahmad, A.L., Sumathi, S., Hameed, B.H. Coagulation of residue oil and suspended solid in palm oil mill effluent by chitosan, alum and PAC. *Chemical Engineering Journal* 118: 99-105, 2006.

Baharuddin, A.S., Abdul Rahman, N.A., Md. Shah, U.K., Hassan, M.A., Wakisaka, M., Shirai, Y. Evaluation of pressed shredded empty fruit bunch (OPEFB)-palm oil mill effluent (POME) anaerobic sludge based compost using fourier transform infrared (FTIR) and nuclear magnetic resonance (NMR) analysis. *African Journal of Biotechnology* 10(41): 8082-8089, 2011.

Baharuddin, A.S., Lim, S.H., Md. Yusof, M.Z.,. Abdul Rahman, N.A., Md. Shah, U.K., Hassan, M.A., Wakisaka, M., Sakai, K., Shirai, Y. Effect of palm oil mill effluent (POME) anaerobic sludge from 500 m^3 of closed anaerobic methane digestion tank on pressed-shredded empty fruit bunch (OPEFB) composting process. *African Journal of Biotechnology* 9(16): 2427-2436, 2010.

Baharuddin, A.S., Wakisaka, M., Shirai, Y., Abd-Aziz, S., Abdul Rahman, N.A., Hassan, M.A. Co-composting of empty fruit bunches and partially treated palm oil mill effluent in pilot scale. *International Agricultural Research* 4(2): 69-78, 2009.

Chang, L.C., Abdullah Sani, A.R., Basran, Z. An economic perspective of oil extraction rate in the oil palm industry of Malaysia. *Oil Palm Industry Economic Journal* 3(1): 25-31, 2003.

Department of Environment Malaysia (DOE)., *Industrial Processes and the Environment (Handbook No 3) Crude Palm Oil Industry*, Kuala Lumpur, 1999.

Foo, K.Y., Hameed, BH. Value-added utilization of oil palm ash: A superior recycling of the industrial agricultural waste. *Journal of Hazardous Materials* 172: 523-531, 2009.

Gutiérrez, L.F., Sánchez Óscar, J., Cardona, C.A. Process integration possibilities for biodiesel production from palm oil using ethanol obtained from lignocellulosic residues of oil palm industry. *Bioresource Technology* 100(3): 1227–1237, 2009.

Mohd Ishak, Z.A., Aminullah, A., Ismail, H., Rozman, H.D. Effect of silane-based coupling agents and acrylic acid based compatibilizers on mechanical properties of oil palm empty fruit bunch filled high-density polyethylene composite. *Journal of Applied Polymer Science* 68(13): 2189-2203, 1998.

Najafpour, G.D., Zinatizadeh, A.A.L., Mohamed, A.R., Hasnain Isa, M., Nasrollahzadeh, H. High-rate anaerobic digestion of palm oil mill effluent in a up-flow anaerobic sludge fixed-film bioreactor. *Process Biochemistry* 41: 370-379, 2006.

National Key Economic Area, Performance Management and Delivery Unit (PEMANDU), http://etp.pemandu.gov.my/ETPedia-@-NKEA_-_National_Key_Economic_ Area .aspx, (1 December 2012)

Paepatung N., Nopharatana, A., Songkasiri, W. Bio-methane potential of biological solid materials and agricultural wastes. *Asian Journal on Energy and Environment* 10(01): 19-27, 2009.

Prasertsan, S., Prasertsan, P. Biomass residues from palm oil mills in Thailand: an overview on quantity and potential usage. *Biomass Bioenergy* 11(5): 387-395, 1996.

Rozman H.D., Peng, G.B., Mohd. Ishak, Z.A., The effect of compounding techniques on the mechanical properties of oil palm empty fruit bunch–polypropylene composites. *Journal of Applied Polymer Science* 70(13): 2647-2655, 1998.

Sulaiman, A., Tabatabaei, M., Hassan, M.A., Shirai, Y. The effect of higher sludge recycling rate on anaerobic treatment of palm oil mill effluent in a semi-commercial closed digester for renewable energy. *American Journal of Biochemistry and Biotechnology* 5(1): 1-6, 2009.

Tan, I.A.W.,. Ahmad, A.L., Hameed, B.H. Adsorption isotherms, kinetics, thermodynamics and desorption studies of 2,4,6-trichlorophenol on oil palm empty fruit bunch-based activated carbon. *Journal of Hazardous Materials* 164(2-3): 473–482, 2009.

Wu, T.Y., Mohammad, A.W., Md. Jahim, J., Anuar, N. A holistic approach to managing palm oil mill effluent (POME): Biotechnological advances in the sustainable reuse of POME. *Biotechnology Advances* 27: 40-52, 2009.

Yahya, A., Sye, C.P., Ishola, T.A., Suryanto, H. Effect of adding palm oil mill decanter cake slurry with regular turning operation on the composting process and quality of compost from oil palm empty fruit bunches. *Bioresource Technology* 101: 8736–8741, 2010.

Zinatizadeh, A.A.L., Mohamed, A.R., Najafpour, G.D., Hasnain Isa, M., Nasrollahzadeh, H. Kinetic evaluation of palm oil mill effluent digestion in a high rate up-flow anaerobic sludge fixed-film bioreactor. *Process Biochemistry* 41: 1038-1046, 2006.

FUEL - 03

Expression of β-Glucosidae Gene From Deep Sea Metagenome of Kawio Archipelago North Sulawesi

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ABSTRACT - Deep sea Research is an Indonesian investment to explore the potential of marine biodiversity that can be developed, so that it can contribute to answering the needs of food, medicine, and renewable energy. This study aims to express β-glucosidase genes from deep sea metagenome of Kawio archipelago, North Sulawesi. A metagenomic approach was conducted to isolate and analyze genes of microorganisms directly from the environment without prior culturing process. Clone of metagenomic DNA p15 and p17 were inserted into pET-32b expression vector and used to transform *E.coli* BL21 (DE3). Gene expression was induced with 1 mM IPTG for 3 hours. Protein BLAST results showed that Bglp_15 dan Bglp_17 has highest homology with β-glucosidase from *Shewanella frigidimarina* NCIMB 400 (Positive 95% and 96%) and hypothetical protein GOS_1944001 [Marine Metagenome] (Positive 78% and 79%). Phylogenetic analysis showed that Bglp_15 and Bglp_17 were related to thermostable beta-glucosidase of *Thermobispora bispora* and beta-glucosidase of uncultured bacterium. Bglp_15 enzyme specific activity was optimum at 60°C and pH 9.4, while Bglp_17 enzyme specific activity was optimum at 60°C and pH 9. Bglp_15 and Bglp_17 were relatively stable after being heated for 4 hours at 60°C. It can be concluded that the β-glucosidase genes isolated from deep sea metagenome of Kawio archipelago are thermostable.

Keywords: β-Glucosidase, Deep sea, Metagenomic, Termostable

FULL PAPER WITHDRAWED by AUTHOR

A New Method for Producing Bioethanol from the Lignocellulose of Meranti bakau by Enzymatic Saccharification and Fermentation

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ABSTRACT - Several papers reported various technical aspects of lignocellulosic bioethanol production. Recalcitrant to saccharification is a major limitation for conversion of lignocellulosic biomass to ethanol. The biological process for converting lignocellulose to fuel ethanol includes delignification to liberate cellulose and hemicelluloses, depolymerization of carbohydrate polymers to produce free sugars and sugars fermentation to produce ethanol. Accesibility of plant cell wall polyscharides to chemical, enzymatic and microbial digestion is limited by many factors, including the presence of lignin and hemicellulose that cover cellulose microfibril. Effort to support fuel ethanol industry fermentation using Indonesian woody plant species Meranti bakau (*Shorea uliginosa* Foxw.) has been developed in an established efficient bioethanol production. This paper relates to a new method for producing bioethanol from the lignocellulose of Meranti bakau by saccharification and fermentation of xylem. As a new method for producing bioethanol, literature study on previous researches of cellulose hydrolysis is necessary. Therefore, the objective of this study is to gain deeper undestanding on the degradation mechanisms of cellulose by enzymes through studies of previous researches which are then compared to the new method of this invention.

Keywords: enzymatic hydrolysis; ethanol; lignocelluloses; Meranti bakau; xylem.

FULL PAPER ACCEPTED by ITB JOURNAL

Oleaginous Yeast with Cmc-Ase Activities For Biofuel Production

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Abstract. The objective of study was to evaluate the possibility of using carboxymethyl cellulose (CMC) as carbon sources for lipid accumulation by oleaginous yeasts : *Candida* sp. (Y09GS34 and Y09GS48) and *Lipomyces* sp. (10381) obtained from InaCC, Indonesia. To obtain the effect of nitrogen on lipid accumulation, the growth medium were formulated as 1% CMC and N limited with 2% CMC and the cultures were incubated for 7 days. Lipid accumulation in the cells was determined by Suddan Black method. and it's composition was identified by Gas Chromatography Mass Spectrometry (GC-MS). During the incubation, the CMC-ase activities were monitored to determine hydrolyses activities by oleaginous yeasts. The results of the study showed that cellulase activities of all strains tested (Y09GS34, Y09GS48, and 10381) was higher in N limited with 2% CMC, namely 1.193, 0.633 and 1.233 unit per hour, respectively. Y09GS34 showed highest lipid accumulation (63.75% per cell dry weight). We identified lipid composition of Y09GS34 was palmitic acid, stearic acid, linoleic acid, and oleic acid; implying that cellulose could be used as carbon source for lipid accumulation by *Candida* sp. (Y09GS34). Therefore, cellulose can be used as carbon source for lipid accumulation by oleaginous yeasts, and Y09GS34 is expected to be potential microbes for bio-fuel research.

Keywords: *cellulose, CMC*; *lipid accumulation; oleaginous yeasts*

FULL PAPER ACCEPTED by ITB JOURNAL

Cassava Pulp Hydrolysis under Microwave Irradiation with Oxalic Acid Catalyst

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ABSTRACT - Microwave irradiation is an alternative method of starch hydrolysis that offers rapid process. The aim of this research was to improve microwave-assisted hydrolysis of cassava pulp ~~by~~ using oxalic acid as a catalyst. Suspension of cassava pulp in 0.5% oxalic acid (1 g/20 mL) was subjected to microwave irradiation at 140-230°C for 5 minutes with 4 minutes of pre-heating. Into some suspensions 1 g of activated carbon was added and they were subjected to the same conditions of microwave irradiation. The soluble fraction of the hydrolysates was analyzed for its total soluble solids, malto-oligomer distribution, glucose content, pH value, and formation of brown compounds. The effects of combined severity parameter at substrate concentration of 5-12.5% on the glucose yield were also evaluated. The highest glucose yield (78% of dry matter) was obtained after hydrolysis at 180°C without activated carbon addition. Heating above 180°C reduced glucose yield and increased pH and formation of brown compounds. The use of activated carbon in microwave-assisted acid hydrolysis of cassava pulp reduced the glucose yield, but suppressed the formation of brown compounds. The highest glucose yield (70-80% of dry matter) was attained at the severity parameter of 1.3-1.5.

Keywords: activated carbon; cassava pulp; glucose; hydrolysis; microwave; oxalic acid; severity parameter.

FULL PAPER ACCEPTED by ITB JOURNAL

Litsea cubeba essential oil yield harvested from different site types in Mt. Papandayan, West Java Indonesia

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ABSTRACT - The objective of this research is to determine the yield and composition of essential oil of *Litsea cubeba* harvested from different site types (habitat) in Mount Papandayan, West Java, Indonesia. Methods used were determining plots of samples at each habitat, followed by laboratory test. Leaf samples were taken from each sampling plot to be tested in a laboratory using steam distillation which was followed by GCMS analysis. The results showed that the yield of essential oil is high (2.76 – 9.33%), and the dominant chemical content found were Eucalyptol (16.97 – 55.78%), α-Terpinenyl acetate (7.27 – 20.44%) and Sabinene (14.45 – 68.05%). This result is in accordance with other research result to support bio-industry sector, especially in pharmacy or health industries.

Keywords: composition, essential oil, *Litsea cubeba*, site types, varieties

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Stability of cassava promising clones for high yield using AMMI model

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Abstract - The aim of this research was to know the stability of clones, tuber yield and ethanol yield of promising clones. The evaluations were done during two years (2007/2008 and 2009) in Lumajang (East Java), and Banyuwangi (East Java), Lampung Selatan (Lampung), Lampung Timur (Lampung) and Lampung Tengah (Lampung). The experiments were done using a randomized complete block design, three replications. The plot size was a 5 m x 5 m. Plants distance was 100 cm x 80 cm. Doses of fertilizers was 93 kg N+ 36 kg P_2O_5 + 60kg K_2O/ha. The clones used were CMM 99008-3, OMM 9908-4, OMM 9904-70, CMM 99023-12, OMM 9904-111, CMM 99023-4 and MLG 10311 as promising clones, as well as Adira 4 and UJ5 (released varieties) as control parameter recorded was fresh tuber yield (t/ha) of nine months old plants. The study showed that based on stability analysis of Additive Main Effects and Multiplicative Interaction model, CMM 99008-3, OMM 9908-4, OMM 9904-70, CMM 99023-4, UJ5, and MLG 10311 and Adira 4 were stable clones, but CMM 99023-12, OMM 9904-111, were not stable clones. Mean of fresh tuber yield of OMM 9908-4 over locations and years was the highest (42,223 t/ha), 15% higher than UJ5 /Kasetsart 50), equal to Rp 4.460.800,-/ha or around US $ 496, - if US $ 1, = Rp 9000,-.

Keywords: use cassava, ethanol, fresh tuber yield, high yield, promising clone, stability

Introduction

Cassava production can be increased by intensification and extensification. Intensification can be done by cultivating high yielding varieties. There are eleven released varieties in Indonesia, such as UJ5 and Adira 4. UJ5 has been developed in Lampung and released in 2000 while Adira 4 is popular varieties of Java and released in 1987.

Recently, there is increasing demand on cassava. One of main reason is application of cassava as raw material for ethanol production. Ethanol industry uses fresh tuber, chips, and starch made of cassava. Supriyanto (2006), and Broto and Richana (2006) reported that to 6 kg fresh cassava tuber is needed to produce 1 liter of ethanol, and it highly affected by variety of cassava.

In Indonesia, cassavas are planted in various environments. Thus, variety trial is should be done on some environment during some planting season. During variety trial, interaction between genotype and environment will acquired which is necessary to assess the the stability of genotype of tested varieties (Sholihin, 2009; Sundari *et. al.*, 2010; Lestari *et. al.*, 2011; Sholihin, 2011). Some promising clones have been identified that resulted from previous cassava breeding activities. These clones are needed to be tested in various locations/environments conditions before releasing the superior ones as new varieties.

Principally, there are two model that can be used in analysis of interaction of genotype and environment, additive models and AMMI (Additive Main Effects and Multiplicative Interaction) model. Many researcher used additive models for analysis of stability. Gauch (1992) proposed model AMMI (Additive Main Effects and Multiplicative Interaction) for analysis of interaction of genotype and environment. Advantage of the AMMI model is no need an assumption that there are a strong linear relationship between variety performance and environmental factors (McLaren & Chaudhary, 1996).

Materials and methods

The evaluations were done during two years (2007/2008 and 2009) in East Java Province (Lumajang dan Banyuwangi) and Lampung Province (Lampung Selatan, Lampung Timur dan Lampung Tengah). The experiments were done using a RCBD (randomized complete block design) with three replications. The plot size was a 5 m x 5 m. Plants distance was 100 cm x 80 cm. Doses of fertilizers was 93 kg N+ 36 kg P_2O_5 + 60kg K_2O/ha. During this study, 9 clones were tested with Adira 4 and UJ5 as control (Table 1.). Parameter recorded was fresh tuber yield (t/ha) after 9 months of plantation. Tuber yield was analyzed using MSTAT (Michigan Statistic), version C software (released by Michigen State University) to obtain the combined analysis of variance. IRRISTAT (International Rice Research Institute Statistic) software, version 5.0 (released by International Rice Research Institute) was used to analyze of variance based on AMMI model, and IPCA (Interaction Principal Component Analysis) score. IPCA scores is $\Sigma_n\lambda_n\gamma_{gn}\delta_{en}$; λ_n = the singular value for PCA axis n; γ_{gn} = the genotype eigenvector for axis n; δ_{en} = the environment eigenvector.

Table 1. Nine clones used in this experiment.

No	Clone	Location of hybridization	Source	Remarks
1	**CMM 99008-3**	ILETRI	ILETRI	Promising clone
2	**OMM 9908-4**	ILETRI	ILETRI	Promising clone
3	OMM 9904-70	ILETRI	ILETRI	Promising clone
4	CMM 99023-12	ILETRI	ILETRI	Promising clone
5	OMM 9904-111	ILETRI	ILETRI	Promising clone
6	CMM 99023-4	ILETRI	ILETRI	Promising clone
7	MLG 10311	UNKNOWN	ILETRI	Promising clone
8	UJ5	KU	KU	Released variety
9	ADIRA 4	ILETRI	ILETRI	Released variety

Note: CMM = cross manihot Malang

MLG = Malang
ILETRI = Indonesian legume and Tuber Crop Research Institute
KU = Kasetsart University

Results and discussion

The result showed that interaction between clones and locations significantly affected fresh tuber yield in 9 months (Table 2). It is natural law that genotype interacts with environment to produce phenotype (Sholihin, 2009; Kalkani & Sharma, 2010; Sholihin, 2011).

Table 2. Combined ANOVA for 9 cassava clones, 4 locations and two years for tuber yield.

Source	Degree of Freedom	Mean Square
Environment (E)	7	37.047**
Error (a)	16	47.237
Clones (C)	8	233.371**
C x E	56	91.954**
Error (b)	128	23.807
Coefficient Variation (%)		13.17

** = 1 % significantly different

On Table 3, it can be seen that the tuber yield rank of each clone was not same each location. Tuber yield of OMM 9908-4 was the first rank in two location, Lumajang (2009) and Lampung Tengah (2009), second rank in Lumajang (2007/2008) and LampungTimur (2007/2008). Tuber yield of OMM 9908-4 was significantly higher than UJ5 in Lampung Tengah (2009), while in the others locations, tuber yield of OMM 9908-4 was equal to UJ5. The mean of the fresh tuber yield of OMM 9908-4 over locations and years was the highest (42,223 t/ha, 15 % higher than UJ5 /Kasetsart 50), equal to Rp 4.460.800,-/ha or around US $ 496, - if US $ 1, = Rp 9000,-. The yield of this clone will be higher if this clone is planted in better environment with high input. Sholihin *et al.* (2010) reported Malang 6 produced more than 100 t/ha when this variety was planted with plant distance 1,25 x 1,25 m and fertilized 500 kg Phonska, 300 kg Urea, and manure ferlilizer 5 t/ha. Doses of fertilizers used in this experiment was 93 kg N+ 36 kg P_2O_5 + 60kg K_2O/ha.

Table 3. Fresh tuber yield (t/ha) of cassava clones/varieties on eight environment, 2007/2008 and 2009.

No	Clone/variety	Tuber yield (t/ha)								
		Lumajang 2007/8	Lampung Selatan 2007/8	Lampung Tengah 2007/8	Lampung Timur 2007/8	Lumajang 2009	Banyuwangi 2009	Lampung Selatan 2009	Lampung Tengah 2009	Mean
1	**CMM 99008-3**	**36.32 a**	**31.72 abc**	**33.33 de**	**25.23 de**	**46.87 a**	**29.81 b**	**30.31 a**	**23.63 e**	**32.15 e**
2	**OMM 9908-4**	**42.09 a**	**35.66 ab**	**40.80 bcd**	**42.55 a**	**60.37 a**	**47.34 a**	**34.13 a**	**34.84 a**	**42.22 a**
3	OMM 9904-70	41.80 a	37.74 a	40.33 bcd	42.66 a	52.81 a	43.76 a	29.65 a	25.41 cd	39.27 bc
4	CMM 99023-12	36.97 a	27.55 bc	27.08 e	35.36 abc	55.18 a	52.19 a	35.52 a	24.49 de	36.79 cd
5	OMM 9904-111	48.41 a	23.73 c	36.72 cd	19.46 e	48.80 a	46.74 a	24.27 a	26.00 c	34.27 de
6	CMM 99023-4	38.41 a	32.52 abc	50.17 a	29.72 cd	54.43 a	30.96 b	30.76 a	17.85 h	35.60 d
7	MLG 10311	33.51 a	39.81 a	49.31 ab	41.63 a	52.95 a	47.52 a	36.79 a	21.56 f	40.38 ab
8	UJ5	40.68 a	37.74 a	43.06 abc	38.42 ab	45.73 a	30.76 b	34.55 a	19.14 g	36.26 cd
9	ADIRA 4	39.55 a	27.62 bc	37.50 cd	32.14 bcd	48.32 a	43.68 a	32.52 a	29.57 b	36.36 cd
Mean		39,75	32.68	39.81	34.13	51.72	41.42	32.06	24.72	37.04
C.V. (%)		14,03	14.15	12.54	11.25	11.48	14.99	13.53	9.63	

Note: The numbers in same colms with same letters are not significantly different at 5% level.

Red mites are an important insect pest in Cassava specially in Java. Farmers in Java do not like UJ3 because Uj3 is susceptible to red mite and tuber disease cause by *Fusarium Sp.* In order to make new variety acceptable by farmer, new variety should not be susceptible to red mite and tuber disease. It was reported that OMM 9908-4 was moderately resistant to mite and to tuber disease caused by *Fusarium* sp, (Sholihin, 2013).

Many industrial products are made from cassava, such as starch, sorbitol, fructose, glucose, crackers, and ethanol. Prospect of ethanol industry is good. The important thing in ethanol industry is supply of raw material. A good raw material is important in determining a good ethanol industry. It was reported that the yield potential of ethanol of OMM 9908-4 was 14472 liter/ha, 20 % and 33 % higher than Adira 4 dan UJ5 (Sholihin, 2013).

It can be seen from the Table 4 that effect of clones x locations interaction was different significantly at 1%, with AMMI models, source of variance of clones x locations interaction can be divided to some components, i.e. IPCA1, IPCA2, IPCA3, and IPCA4. IPCA 1 and IPCA 2 were different significantly, while IPCA 3 and IPCA 4 was not significantly difference. Forty eight percent of interaction sum square was contributed by IPCA1, 26 % by IPCA 2, the residual was by IPCA 3 and IPCA 4.

Table 4. Analysis of variance based on AMMI model for tuber yield

Source of variance	Degree of freedom	Mean squares
Location (L)	7	37.047**
Error	16	47.237
Clone (C)	8	233.371**
C x L	56	91.954**
IPCA1	14	63.2134**
IPCA2	12	39.7074*
IPCA3	10	19.2021
IPCA4	8	10.3518
Combined error	128	23.807

**, *: significantly different at 1 % and 5 %, respectively

On average, tuber yield of OMM9908-4 was the highest, while CMM99008-3 was lowest. Based on Figure 1, it can be determined that clone which was not on one point on the vertical axis means those clones had a different main effect. The tuber yield of clone 8 (UJ5) and 9 (Adira 4) were similar, but interaction effect with location was different. Clone 8 (UJ5) has positive interaction with location D, while clone 9 (Adira 4) has negative interaction with D location.

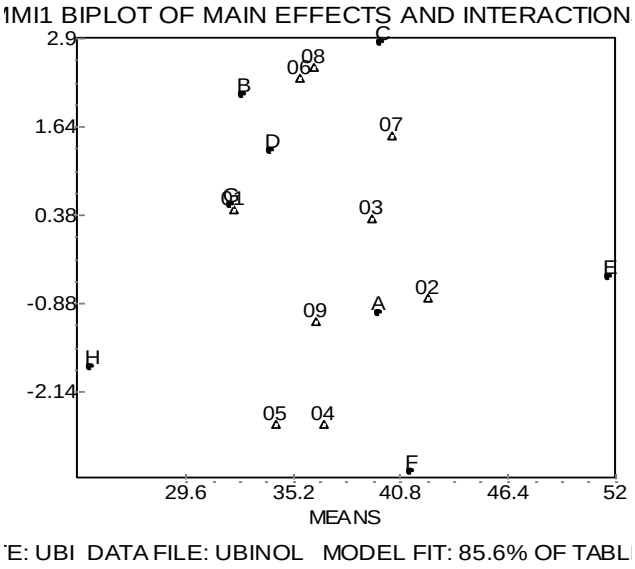

Figure 1 Biplot of IPCA 1 and fresh tuber yield in nine months

Note:

1. CMM99008-3
2. OMM9908-4
3. OMM9904-70
4. CMM99023-12
5. OMM9904-111
6. CMM99023-4
7. MLG 10.311
8. UJ5
9. ADIRA 4

A: Lumajang, 07/8, B: Lampung S., 07/8,
C: Lampung T., 07/8, D: Lampung T, 07/8
E: Lumajang, 09 F: Banyuwangi, 09
G: Lampung S., 09 H: Lampung T., 09

IPCA score for four locations during two years and mean of tuber yield is given in Table 5. Biplot IPCA 1 and IPCA 2 for Environment based on tuber yield were given in Figure 2. Based on this figure, it can be seen that locations used was a good enough because location were varied. Position of A (Lumajang, 07/08) was far from C (Lampung T., 07/08), H (Lampung T., 09), E (Lumajang, 09), G (Lampung S. 09), B (Lampung S., 07/08), and D (Lampung T, 07/08). These mean the environments were varied.

Table 5. IPCA score for locations and mean of fresh tuber yield in nine months.

Locations	Fresh tuber yield t/ha	IPCA1	IPCA2
A.Lumajang,Inceptisol, 110 m above sea level, 2007/2008	39,75	-1.034	2.765
B. Lampung Selatan, Ultisol,135 m above sea level, 2007/2008	32.68	2.068	-0.447
C. Lampung Timur, Ultisol, 2007/2008	39.81	2.831	1.782
D. Lampung Tengah, Ultisol, 58 m above sea level, 2007/2008	34.13	1.280	-2.889
E. Lumajang,Inceptisol, 110 m above sea level, 2009	51.72	-0.532	-0.027
F. Banyuwangi,Entisol, 168 m above sea leavel, 2009	41.42	-3.302	-1.229
G. Lampung Selatan, Ultisol, 135 m above sea level, 2009	32.06	0.507	-0.669
H. Lampung Tengah, Ultisol, 58 m above sea level, 2009	24.72	-1.818	0.713

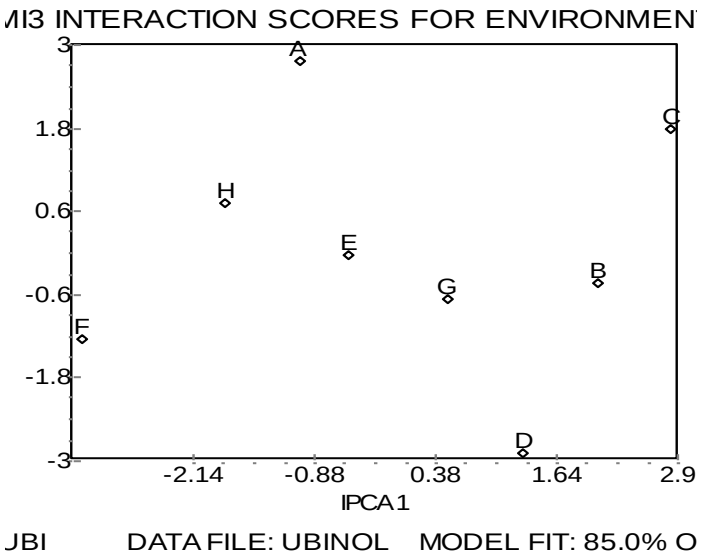

Figure 2. Biplot of IPCA 1 and IPCA 2 for environment based on fresh tuber yield in nine months.

Note:
A: Lumajang, 07/8, B: Lampung S., 07/8, C: Lampung T., 07/8, D: Lampung T, 07/8
E: Lumajang, 09 F: Banyuwangi, 09 G: Lampung S., 09 H: Lampung T., 09

The average of tuber yield of OMM 9908-4 was the highest, followed by MLG 10311, OMM 9904-70, CMM 9904-100, Adira 4, and UJ5. UJ5 have high score on IPCA 1 (2.487) and CMM 99023-12 had low score (-2.603) (Table 6). Biplot of IPCA1 and IPCA2 for clones based on tuber yield was given in Figure 3. Based on this figure, it can be identified the stability of clone which located near point (0,0) considered as stable clone. Clone OMM 9904-70, CMM 99008-3, OMM 9908-4, and Adira 4 were more stable than CMM 99023-4, MLG 10311, and UJ5. There are two possibilities to explain stability of clone. High stability could be caused (i) clone is a hybrid and (ii) it has a genetic potential to perform well irrespective of the environment where they are grown. Sholihin (2011) reported that environmental factors which important in determining stability of the tuber yield in cassava clones were soil pH on Subsoil, the maximum air temperature first month, the minimum relative humidity sixth month, The total of rain fall ninth months, and the minimum air temperature third months.

Table 6. IPCA score for clones and fresh tuber yield in nine months.

No.	Clones	tuber yield (t/ha)	IPCA1	IPCA2
1	CMM 99008-3	32.15	0.465	0.911
2	OMM 9908-4	42.22	-0.813	-0.875
3	OMM 9904-70	39.27	0.335	-0.967
4	CMM 99023-12	36.79	-2.603	-2.173
5	OMM 9904-111	34.27	-2.598	2.953
6	CMM 99023-4	35.60	2.342	1.663
7	MLG 10311	40.39	1.513	-1.747
8	UJ5	36.26	2.487	0.060
9	Adira 4	36.36	-1.129	0.174

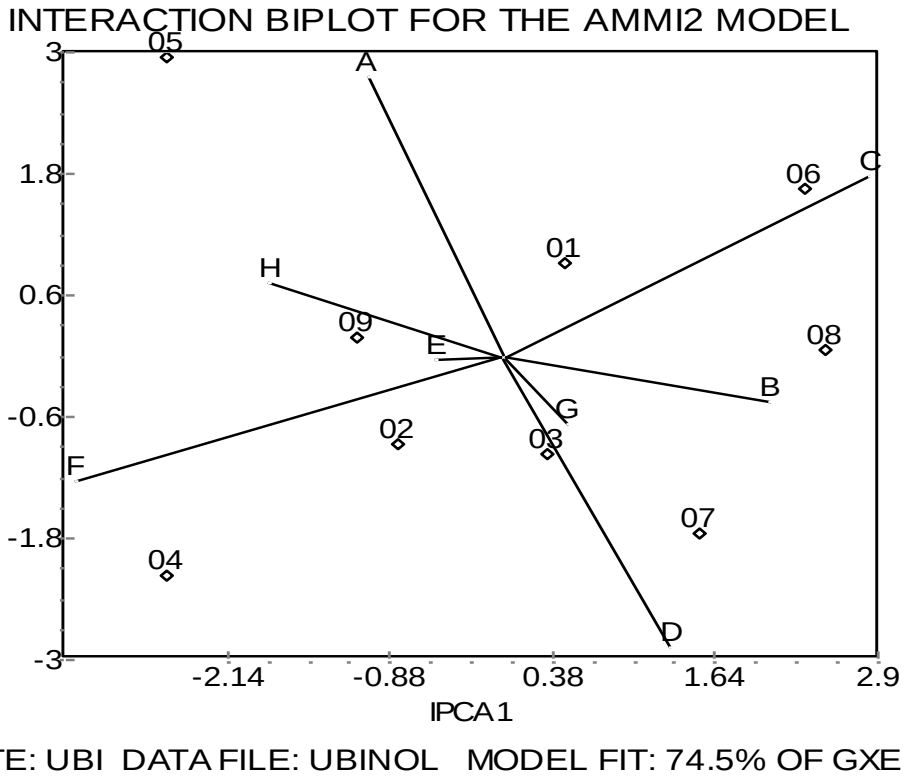

Figure 3. Biplot of IPCA 1 and IPCA 2 for clones based on fresh tuber yield in nine months

Note: 1. CMM99008-3
2. OMM9908-4
3. OMM9904-70
4. CMM99023-12
5. OMM9904-111
6. CMM99023-4
7. MLG 10.311
8. UJ5
9. ADIRA 4

A: Lumajang, 07/8 B: Lampung S., 07/8 C: Lampung T., 07/8, D: Lampung T, 07/8
E: Lumajang, 09 F: Banyuwangi, 09 G: Lampung S., 09 H: Lampung T., 09

Conclusion

1. Clone OMM 9904-70, CMM 99008-3, OMM 9908-4, and Adira 4 were more stable than CMM 99023-4, MLG 10311, and UJ5.
2. The mean of the fresh tuber yield of OMM 9908-4 over locations and years was the highest.

References

Supriyanto, Prospek pengembangan industri bioetanol dari ubikayup, Proseding lokakarya prospek, strategi, dan teknologi pengembangan ubikayu untuk agroindustri dan ketahanan pangan, Harnowo, Subandi and Saleh (eds.), pp.88-95, 2006.

Broto, W., Richana, N. Inovasi teknologi proses industri bioetanol dari ubikayu skala pedesaan. Proseding lokakarya prospek, strategi, dan teknologi pengembangan ubikayu untuk agroindustri dan ketahanan pangan, Harnowo, Subandi and Saleh (Ed.), pp.60-73, 2006.

Sholihin, The genotypes x environment interaction for starch yield in nine months of cassava promising clones. *Indonesian Journal Agricultural Science* 10(1): 12-18, 2009.

Sholihin, AMMI model for interpreting clone - environment interaction in starch yield of cassava. *HAYATI Journal of Bioscience* 18 (1): 21-26, 2011.

Sundari T, Noerwijati, K., Mejaya, I.M.J. Hubungan antara komponen hasil dan hasil umbi klon harapan ubikayu. *Jurnal Penelitian Pertanian Tanaman Pangan* 29 (1): 29-35, 2010.

Kalkani R.K., Sharma, Y. Genetic component analysis for yield and yield contributing traits under diverse environments in barley. *SABRAO Journal of Breeding and Genetics* 42 (1): 9-20, 2010.

Lestari, A.P., Abdullah, B., Junaedi, A., Aswidinnoor, H. Performance of grain quality and aroma of aromatic new plant type promising rice lines. *Indonesian Journal Agricultural Science* 12(2): 84-93, 2011.

Gauch H.G. Statistical Analysis of Regional Yield Trial, Elsevier Science publishers Amsterdam, pp. 1-278, 1992.

McLaren, C.G., Chaudhary, R.C. Use of additive main effects and multiplicative interaction models to analyze multilocation rice variety trials, pp. 1-9, 1996, (unpublished).

Sholihin, Saleh, N., Radjit, B.S., Sundari, T., Wahyuni, T.S., Mejaya, I.M.J. Perakitan varietas dan perbaikan sistem produksi ubikayu umur genjah sesuai untuk pangan dan industri dengan potensi hasil 40 - 60 t/ha, Laporan Akhir RPTP/RDHP Tahun Anggaran 2010, Balitkabi, pp. 1-107, 2010.

Sholihin, Varietas litbang UK 2, hasil tinggi dan sesuai untuk bioethanol, Prosiding seminar nasional, akselerasi pembangunan pertanian berkelanjutan menuju kemandirian pangan dan energy, Purnomo, Harisudin, Praseptiangga, Magna, Rahayu, Widiyanto, Indreswari, Yanti dan Hertanto, (eds.), pp. 565-569, 2013.

Sholihin, Analisis interaksi genotipe x lingkungan untuk hasil ubi ubikayu dengan model AMMI, Proseding seminar nasional, Pemuliaan berbasis potensi dan kearifan lokal menghadapi tantangan global, Peripi Komda Banyumas, Agung, Suwarto, Sasongko, Susanto, Winanto dan Riyanto, (eds.), pp.510-520, 2011.

The Effect of Stimulant Compound to Biogenic Methane Formation and Dynamics of Bacterial Population in Coal Bed Methane

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ABSTRACT - Coal Bed Methane (CBM) is a renewable energy that produced through the thermogenic and biogenic activity during the process of coal formation. Biogenic methane formation occurs through a process conducted by indigenous methanogens via methanogenesis and degradation of organic molecules by syntrophic bacteria from coal. Methanogenesis process can occur through three pathways that are i). CO_2 reduction, ii). Acetate fermentation, and iii).Methanol or methylamine dismutation. In this research, methane formations were stimulated using a simple carbon source in a microcosm. The microcosm set-up used subbituminous coals at a temperature of 37^{o}C in anaerobic chamber. Stimulation process was carried out for 54 days and was observed on days 2, 15, 24, 45, and 54 . The addition of stimulants showed the difference of initial pH of basal medium, pH 8.95 without stimulation, 7.76 for Na-asetat stimulant, 6.69 for methanol stimulant, and 4.06 Formic acid stimulant. Gas Chromatography analysis showed the highest methane recovery value of 5,034 mmol/g coal produced from stimulants Na-acetate at 28 days incubation time. The methane gas production was five times higher than the production of methane gas without giving stimulants at the same time. Changes in microbial communities due to stimulants was observed through Denatured Gradient Gel Electrophoresis methode. Product size of 180 pb from PCR amplification using primers 338F-GC and 518R (the universal bacterial 16S rRNA primers) were separated by DGGE method on 10% polyacrylamide gel with a 35-60% denaturant for 10 hours. DGGE results showed a change in the bacterial community when given stimulants.

Keywords: Coal Bed Methane, Denatured Gradient Gel Electrophoresis, Methanogenesis, Stimulations, Volatile Fatty Acids.

FULL PAPER ACCEPTED by ITB JOURNAL

Bacterial Community Structure of Planktonic Cells and Biofilm at Saguling Hydro Power using Denaturing Gradient Gel Electrophoresis (DGGE)

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ABSTRACT – Bacterial communities in planktonic cells and biofilm at Saguling Hydro power was investigated using denaturing gradient gel electrophoresis (DGGE) technique. Physico-chemical characteristic of aqueous medium indicated that the water source was classified as moderately polluted (eutrophic). The Mann-Whitney U tests showed that there was no significantly difference between the bacterial community of planktonic cells and biofilm. The 16S rRNA sequences revealed that bacteria recovered from the planktonic cells were affiliated with Betaproteobacteria and Bacteroidetes phyla. Meanwhile, the sequences bacteria revealed from biofilm were closed to Betaproteobacteria, Alphaproteobacteria, and Gammaproteobacteria groups. Overall, the majority of microbes identified from the two samples were belongs to Betaproteobacteria group.

Keywords: bacterial community structure, biocorrosion, biofilm, DGGE, planktonic cells, Saguling

FULL PAPER WITHDRAWED by AUTHOR

The cytotoxic poliketides of an endophytic fungus *Mycoleptodiscus indicus* against T47D cells isolated from Indonesian medicinal plant *Thyponium divaricatum* L.Decne

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ABSTRACT - The current study was to investigate cytotoxic metabolites that produced by an endophytic fungus from Indonesian medicinal plant, *Thyponium divaricatum* L. Decne. The fungus endophytic was fermented in Potato Dextrose Broth for 20 days at room temperature and extracted with hexane, dichloromethane, ethyl acetate, butanol and methanol. All extracts were tested to against T47D cells with Sulforhodamine B method and showed the viability T47D cells about 49.06-38.11%. The NMR spectroscopy data showed the active extract was belonged to polyketides. At the first time, cytotoxic metabolites of an endophytic fungus *M. indicus* from Indonesia against T47D cells from various extracts was reported.

Keywords: cytotoxic, endophytic fungus, *Mycoleptodiscus indicus, Thyponium divaricatum, T47D cells.*

Introduction

The incidence of breast cancer in Indonesia showed an increase every year and as the second rank incidence after cervical cancer. It also considered the breast cancer is the leading cause of cancer death among female at developing countries, accounting for 23% of the total cancer cases and 14% of cancer death (Jemal *et. al.*, 2011). In Indonesia, based on cancer data collection that there will be at least 170-190 new cancer cases annually for each 100.000 people including breast cancer (Didid & Rukmini, 2002). Now a days, medical treatments in breast cancer are chemotherapy, hormonal treatment, surgical excision, and irradiation. Beside that, various kinds of medicinal plants which have been used to treat breast cancer for a long time as alternative treatments (Pratumvinit *et. al.*, 2009). Several studies have been found out the anticancer agents from medicinal plants as natural products for preventing and treating breast cancer.

One of Indonesian medicinal plants that is widely used as anticancer agents is *Thyponium divaricatum* L. Decne which has synonym with *Thyponium flagelliforme* Lodd. Bl. or called rodent tuber. Rodent tuber belonged to Araceae which has 30 cm height, whitish tuber, triangular leaves, and unique yellowish flower from the root (Lai *et. al.*, 2008). The crown-shaped flowers are small with white long tail similar to rats. But there are some types that have a red-colored petals (Nurrochmad *et. al.*, 2011). Previous study showed the various extracts of the plants could against murine P388 leukemia cells (Choo *et. al.*, 2001), NCI-H23 non-small cell lung carcinoma cell line (Lai *et. al.*, 2008), T4 lymphoblastoid cell line CEM-ss (Mohan *et. al.*, 2008), some pathogenic bacteria and have antioxidant activity (Mankaran *et. al.*, 2013). The phytochemical screening showed the plants contained alkaloids, flavonoids, terpenoids and steroids. The main constituens of the plants were alkaloids and flavonoids (Mankaran *et. al.*, 2013).

The potential medicinal plants associated with microorganism called endophytic. Endophytes are microbial entities that live and spend all or part their lifecycle colonizing in living tissues of plants without causing any harm to them (Ahmed *et. al.*, 2012). Endophytes also have no apparent disease symptoms and produce bioactive metabolites of pharmaceutical (Premjanu and Chellam, 2012) such as anticancer, antimicrobial and antioxidant (Pimentel *et. al.*, 2011). They have symbiotic and mutualistic relationship with the host plants. The bioactive compounds of the plants are used for defense against pathogens. Some of the compounds are sources of novel drugs (Jalgaonwala *et. al.*, 2011). Endophytic are relatively unstudied and a promising source of novel organic natural metabolites exhibiting a variety of biological activities. The simple preliminary analysis of bioactive could be done on phytochemical screening (Tiwari *et. al.*, 2011). Beside that, the biodiversity of the fungi and the phylogenetic still needed analysis based on Internal Transcribed Spacer (ITS) region rDNA (Garcia *et. al.*, 2012). The fungus is identified based on ITS region as *Mycoleptodiscus indicus* UAMH 8520 about 98% and phylogenetic data shows that it is different with M.indicus UAMH 8520 an prob. Therefore, the new strain of microbes as one of mega biodiversity were expected to be natural drug, especially for anticancer medicine.

Results and Discussion

The results of mass yield (mg) of fungi fermentation and cytotoxic activity of extracts with 100 ppm concentration are described in Table 1. The dichloromethane extract showed the best activity against T47D cells with the lowest mass yield (3.30 mg and viability cells 38.11%). The other extracts revealed viability cells 42.64% from butanol, 43.40% from biomass which was extracted with methanol, and 49.06% from hexane. Value IC_{50} of all extracts also showed low cytotoxicity. Generally, all extracts could inhibit cells about 40-50% and indicated low cytotoxic activity. The active extracts should inhibit cells > 70% at 100 µg/mL and IC_{50} (µg/mL) < 100 (Hazalin *et. al.*, 2009; Sundaram *et. al.*, 2011). Phytochmical screening also showed alkaloids was detected in ethyl acetate

and butanol extracts. The other extracts did not contain alkaloids, saponin, phenol, tannin and flavonoids even extracts had cytotoxic activity. In this study, the cytotoxic metabolites of fungus contained cytotoxic alkaloids. Alkaloids and flavonoids are the main phytochemical constituents of *T. flagelliforme* which are found to be in the highest amount in two and four month old of *ex vitro* plants (Mankaran *et. al.*, 2013). A cytotoxic alkaloid ever had been isolated from endophytic Chaetomium sp IFB E-015 (Jiao *et. al.*, 2006). Then, a new cytotoxic cytochalasan alkaloid named chaetoglobosin U was isolated from Endophytic *Chaetomium globosum* IFB-E019 (Ding *et. al.*, 2012). We emphasize that the cytotoxic compounds could isolated from the fungus biomass and metabolites which is secreted by the fungus into media PDB.

Table 1 Cytotoxic activity of endophytic fungus extracts against T47D cells

Crude extracts	Mass yield (g)	Viability cells (%)	IC50 ((µg/mL)	Phytochemical test
Hexane	11.80	49.06	>100	-
Dichloromethane	3.30	38.11	>100	-
Ethyl acetate	12.60	44.53	>100	alkaloids
Butanol	70.40	42.64	>100	alkaloids
Methanol	383.90	43.40	>100	-

We desire to isolated metabolites compounds from ethyl acetate because it contained polar and semipolar compounds. Analysis of NMR spectroscopy suggested the presence of chromone derivative which was belonged to polyketides class. The cytotoxic activity of polyketides is presented in Table 2. The highest (100 ppm) and the lowest (25 ppm) concentration of fungus polyketides showed 33% and 82% viability T47D cells. The cytotoxic activity of polyketides is better than ethyl acetate extracts. It shows that ethyl acetate extract consists a few of pure cytotoxic compounds which make possible to against T47D cells effectively.

Table 2 Cytotoxic activity of polyketides from ethyl acetate extract

concentration (ppm)	viability cells
100	33
75	45
50	67
25	82

For the plant, the cytotoxic activity *of T.divaricatum* L.Decne had been widely exposed. The hexane extract from tubers against P388 murine leukemia cell and showed IC_{50} 15 µg/mL (Choo *et. al.*, 2001). The hexane and dichloromethane of whole plant against NCI-H23 non small cell lung carcinoma cell line and revelaled IC_{50} < 15 µg/mL (Lai *et. al.*, 2008). The antiproliferative effect of some dicholoromethane and ethyl acetate extracts against CEM-ss cell showed citotoxicity about 10.8-5.8 µg/mL from leaves and 6.5-8.2 µg/mL from tubers (Mohan *et. al.*, 2008). The constituents antiproliferative properties of the plants could against breast cancer HS578T cell line and increased photoactivation (Lai *et. al.*, 2010). It considered rodent tuber extract (RTE) could against T47D cells with IC_{50} 632 µg/mL (Nurrochmad *et. al.*, 2011). In our study, there is no study about endophytic isolate from the plant yet.

Previous study, the potential endophytic fungus was identified 98 % as *Mycoleptodiscus* sp. and it could be a new strain of species because only the ITS region was identified. It might be fungi isolates had different protein and needed quite long sequence to identified them. In the meantime, based on

the fact that ITS1 and ITS2 may help align the unknown sequences at least to a genus level, further molecular studies of unclear isolates are urgently needed (Huang *et. al.*, 2012; Orlandelli *et. al.*, 2012). The fungus was belonged to class of Ascomycota, ordo of Magnaporthales and family of Magnaporthaceae. The most fungi frequently isolated and had a lot fungi belonged to Ascomycota. It considered that generally Ascomycetous fungi which are conidial or sterile and that form melanized structures like inter and intracellular hyphae and microsclerotia in the roots of plants (Jalgaonwala *et. al.*, 2011). The fungus also had been ever isolated from *Echinacea purpurea* L and had antifungal and insecticidal activity (Rosa *et. al.*, 2012). Panamanian endophytic fungi *M.indicus* was isolated and evaluated for the antiparasitic and in vitro anticancer activities and showed selective activity. However in this study, we have an endophytic fungus *M.indicus* with new strain produced cytotoxic metabolites against T47D cells that isolated from Indonesian plant. Today, more and more studies have focused on the endophytic fungi extracted from various medicinal plants for their anticancer activity. Several studies on endophytic fungi have obtained noteworthy isolates for synthesising bioactive compounds. Some of these compounds have been used for novel drug discovery.

Experimental

General

Impresinated extracts were performanced using Merck silica gel 7733, stasioner phase using Merck silica gel 7731 and TLC plate using Merck silica gel 60. NMR data were NMR 1D (^{1}H and ^{13}C) and NMR 2D in deuterated chloroform ($CDCl_3$).

Plant Material

The fresh *Thyponium divaricatum* L.Decne was collected from Research Institute of Medicinal Plants and Aromatics (BALITRO), Bogor, Indonesia. The plant was stored at 4^{o}C until been used. The plant was identified by Indonesian Institute of Sciences Research Center for Biology, Cibinong, Indonesia.

Isolation of Endophytic Fungi

Endophytic fungi were isolated from leaves, roots and branches of the plant. The complete fresh plants were washed under running tap water for 10 minutes. For sterilized surface leaves, roots and branches, they were cut into 1 cm length segments. Sample segments were immersed in etanol 70% for 1 minute, sodium hypochloride 5% for 5 minutes, etanol 70% for 30 seconds and water for 30 seconds. The sterilized segments were put into petridish containing CMM and PDA (Hazalin *et. al.*, 2009).

Fermentation and Extraction of Cytotoxic Metabolites

The endophytic fungus *M.indicus* was cultivated on Potato Dextrose Agar plates at 25^{o}C for 7-14 days. Three pieces (0.5 X 0.5 cm) of mycelia agar plugs were inoculated into 1L PDB and incubated at room temperature under agitation 150 rpm for 20 days. After this, the secondary metabolites of the fungus was separated from mycelia by vacuum filtration. The metabolites was extracted with hexane, dichloromethane, ethyl acetate and butanol. Biomass was dried in oven and extracted with methanol. All the solutions were evaporated using rotary vaccum evaporator at 40^{o}C. The crude extracts were analyzed for cytotoxic compounds against T47D cells.

Cytotoxicity assay

For cytotoxic activity, each extracts were dissolved in 100 % DMSO to have stock solutions at 4mg/ml concentration. Final concentration at DMSO 0.5% were achieved by diluting the stock with medium. The cells were maintenanced in DMEM containing L-glutamine supplemented with 10%

v/v fetal bovine serum, sodium bicarbonate, 100 µg/ml streptomycin and 100 U/ml penicillin at 37^{o}C in a incubator of 5% CO_2. The T47D cells had 80% confluence after 3 days incubation. The cells were washed 1 times with 20 ml PBS, added 1 ml Trypsin solution and incubated for 5 min. Then, fresh medium was added into the cell container. In cytotoxicity assay, 190 µl cell solution was mixed with 10 µl test sample solution (in 10 % DMSO) / well and incubated at 37^{o}C for 3 days. Test samples concentrations were 2.5ppm, 5.0ppm, 10ppm, 20ppm, and 40ppm. It used cisplatin as control. The cells were fixed by adding 50 µl TCA, incubated at 4^{o}C for 30 minutes, washed with tap water 4 times and air dried plates until no standing moisture was visible. Plates containing wells were stained with 100 µl SRB in 1% acetic acid at room temperature for 30 minutes. Then, the plates were rinsed off unbound dye with 1% acetic acid for 4 times and air dried. Amount of 100 µl 10mM Tris base (pH.10) was added into each of the wells to solubilize dye on a gyratory shaker for 5 minutes. The optical density (OD) was measured at 515 nm using an ELISA plate reader (Sundaram *et. al.*, 2011). Data were calculated as percentage viability using the following formula (Tanawan *et. al.*, 2010):

$$\text{Inhibition (\%)} = \frac{OD\ (cells+sample) - OD\ (0\ day)}{OD\ (cells+10\%\ DMSO) - OD\ (0\ day)} \text{ X } 100\%$$

Molecular identification

Endophytic fungus was identified based on the sequence of the ITS regions. DNA was isolated from mycelia and the ITS region was amplified by Polymerase Chain Reaction (PCR) with ITS1 and ITS4 primers. The PCR product was purified using the QIAquick PCR Purification kit (Qiagen). BigDye terminator cycle sequencing Ready Reaction Kit (Perkin Elmer Applied Biosystem) was used and the product was purified with the AutoSEQ G-50 Kit (Qiagen). The sequences determined was aligned by the MEGA program (version 5.0) and analysed by Basic Local Alignment Search Tool nucleotide (BLAST)n (Garcia *et. al.*, 2012). Phylogenetic analysis was constructed by the Neighbour Joining (NJ) method with 1.000 bootstrap repetitions (Huang *et. al.*, 2012).

Chemical analysis

First, all crude active extracts were impresinated with stasioner phase and it used silica gel. Then, they were fractionated using liquid vacuum chromatography (LVC). Hexane, hexane : CHCl3 (100 : 100 w/w), CHCl3, and CHCl3 : MeOH (95 : 5 w/w) to (0 : 100 w/w) were used in LVC as elution system. All fractions were carried out on thin layer chromatography (TLC), visualized under UV light and sprayed with anisaldehyde-H_2SO_4 reagent followed by heating. The single spot was isolated using coloumn chromatography with hexane : aceton (10 : 1 w/w) as elution system. The pure compound was analyzed by NMR spectroscopy.

Phytochemical screening

Phytochemical screening were carried out for all active extracts as per standard methods (Tiwari *et. al.*, 2011).

a. Detection of alkaloids: extracts were dissolved in dilute Hydrochloric acid and filtered. Filtrates were treated with Dragendroff's reagents. Red presipitation indicated the presence of alkaloids.
b. Detection of saponin: extracts were diluted with 20 ml dH_2O and shaken for 15 minutes. Formation of 1 cm of foam indicated the presence of saponin.
c. Detection of phenols: extracts were of treated with 3-4 drops of ferric chloride solution. Bluish black colour formation indicated the presence of phenols.

d. Detection of tannin: extracts were added 1% gelation containing sodium chloride. White presipitation indicated the presence of tannin.
e. Detection of flavonoids: extracts were treated with 3-4 drops sodium hydroxide solution. The intense yellow colour which becomes colourless on addition of dilute acid indicated the presence of flavonoids.

Conclusion

An endophytic fungus *M.indicus* with new strain from Indonesian plant *T.divaricatum* L.Decne could produce cytotoxic metabolites against T47D cells. Based on NMR data that the metabolites contained polyketides compounds. The kinds of polyketides as cytotoxic metabolites were recommended for further studies to investigate other biological activities.

Acknowledgements

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References

Ahmed, M., Muzaffer, H., Manoj, K.D., Sanjana, K. Isolation of microbial endophytes from some ethnomedicinal plants of Jammu and Khasmir. *Journal of Natural Product and Plant Resources* 2(2): 215 - 220, 2012.

Choo, C.Y., Kit, L.C., Teng, W.S., Yukio, H, Koichi, T. The cytotoxicity and chemical constituens of the hexane fraction of *Thyponium flagelliforme* (Araceae). *Journal of Ethnopharmacology* 77: 129-131, 2001.

Didid, T, Rukmini, M. Cancer in Indonesia, present and future. *Japanese Journal of Clinical Oncology* S17 – 21, 2002.

Ding, L., Dahse, H.M., Herwech, C. Cytotoxic alkaloids from Fusarium incarnatum associated with the mangrove tree *Aegiceras corniculatum*. Journal of Natural Products **75**(4): 617 – 621, 2012.

Garcia, A., Sandro, A.R., Celso, J.R.F., Celso, V.N, Joao, A.P. Diversity foliar of endophytic fungi from medicinal plant *Sapindus saponaria* L.and their localization by scanning electron microscopy. *Biological Research* 45: 139-148, 2012.

Hazalin, N.A.M.N., Kalavathy, R., Siong, M.L., Ibtisam A.W., Anthony, L.J.C, Abu, B.A.M. Cytotoxic and antibacterial activities of endophytic fungi isolated from plants at the National Park, Pahang, Malaysia. *BMC Complementary and Alternative Medicine* **9**(46): 1-5, 2009.

Huang, W.Y., Cai, Y.Z., Surveswaran, S., Hyde, K.D., Corke, H. & Sun, M. Molecular phylogenetic identification of endophytic fungi isolated from three Artemisia species. *Fungal diversity* 36: 69-88, 2012.

Jalgaonwala, R.E., Bhavna, V.M., Raghunath, T.M. A Review: Natural products from plants associated endophytic fungi. *Journal of Microbiology and Biotechnology Research* 1(2): 21-32, 2011.

Jemal, A., Freddie B., Melissa, M.C., Jacques, F., Elizabeth, W, David, F. Global cancer statistics. CA: A Cancer Journal for Clinicians 61: 69-90, 2011.

Jiao, R.H., Shu, X., Jun, Y.L., Hui, M.G., Hui, D., Chen, X., Hai, L.Z., Ren, X.T. Chaetominine, a Cytotoxic Alkaloid Produced by Endophytic Chaetomium sp. IFB-E015. Organic Letters 8(25): 5709–5712, 2006.

Lai, C.S., Rosemal, H.M.H.M., Nair, N.K., Majid, M.I.A., Mansor, S.M., Navaratnam, V. *Thyponium flagelliforme* inhibits cancer cells growth in vitro and induces: An evaluation by bioactivity approach. *Journal of Ethnopharmacology* 118: 14-20, 2008.

Lai, C.S., Rosemal, H.M.H.M., Nair, N.K., Majid, M.I.A.,Mansor, S.M., Navaratnam, V, Chemical constituents and in vitro anticancer activity of *Thyponium flagelliforme* (Acarceae). *J. Ethnopharmacol*. **127**, pp. 486-494, 2010.

Mankaran, S., Kumar, D., Sharma, D., Singh, G. *Thyponium flagelliforme*: A multipropose plant, *International Research Journal of Pharmacy* 4(3): 45-48, 2013.

Mohan, S., Bustamam, A., Ibrahim, S., Al-Zubairi, A.S., Aspollah, M, Anticancerous effect of *Thyponium flagelliforme* on Human T4 –Lymphoblastoid cell line CEM-ss. *Journal of Pharmacology and Toxicology* 3(6): 449-456, 2008.

Nurrochmad, A., Endang, L., Edy, M. Anticancer activity of rodent tuber (*Thyponium flagelliforme* (Lodd.) Blume on human breast cancer t47d cells. *International Journal of Phytomedicine* 3: 138-146, 2011.

Orlandelli, R.C., Alberto, R.N., Filho, C.J.R., Phampile, J.A. Diversity of endophytic fungal community associated with *Piper hispidum* (Piperaceae) leaves. *Genetics and Molecular Research* 11(2): 1575-1585, 2012.

Pimentel.M.R., Gustavo, M., Ana, P.D., Mario, R.M.J., Glaucia, M.P. The use of endophytes to obtain bioactive compounds and their application in biotransformation process. *Biotechnology Research International* 1-11, 2011.

Pratumvinit, B., Srisapoomi, T., Worawattananon, P., Opartkiattikul, N., Jiratchariyakul, W., Kummalue, T. In vitro antineoplastic effect of *Ficus hispida* L plant against breast cancer cell lines. *Journal of Medical Plants Research* 3: 255-261, 2009.

Premjanu, N., Chellam, J, Endophytic fungi a repository of bioactive compounds-A review. International Journal of Institutional Pharmacy and Life Sciences 2(1): 135-162, 2012.

Rosa, L.H., Nurhayat, T., Natascha, T., David, E.W., Zhigiang, P., Ulrich, R.B., James, J.B., Natasha, M.A., Larry, A.W., Rita, M.M. Diversity and biological activities of endophytic fungi associated with micropropagated medicinal plant *Echinacea purpurea* (L.) Moench. *American Journal of Plant Sciences* 3: 1105 – 1114, 2012.

Sundaram, S., Satish, K.V., Priyanka, D. In vitro cytotoxic activity of Indian medicinal plants used tradisionally to treat cancer. *Asian Journal of Pharmaceutical and Clinical Research* 4(1): 27-29, 2011.

Tanawan, K.M.D., Suporn, F., Weena, J. Antiproliferative and antimicrobial activities of endophytic fungus isolated from *Erycibe elliptilimba*. *Siriraj Medical Journal* 62: 237-240, 2010.

Tiwari, P., Bimlesh, K., Mandeep, K., Gurpreet, K., Harleen, K. Phytochemical Screening and Extraction: A Review. *Internationale Pharmaceutica Sciencia* 1(1): 98-106, 2011.

The natural cytotoxic compounds of endophytic fungi from medicinal plant *Thyponium divaricatum* L.Decne

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ABSTRACT - A total of 14 endophytic fungi isolates were isolated from leaves, branches and roots of medicinal plant *Thyponium divaricatum* L.Decne. All endophytic fungi were tested as cytotoxic agents against T47D cells using SRB method. The invitro assay showed that five endophytic fungi produced cytotoxic compounds. They were Tf.1F, Tf.4F, Tf.8F, Tf.10F, Tf.11F. The active cytotoxic compounds were extracted with n-Hexan, methylene chloride, ethyl acetate and methanol. IC_{50} of active extracts that contained alkaloids were about 7.90 – 58.80µg/mL.

Keywords: cytotoxic, endophytic, fungi, IC_{50}, invitro assay, *Thyponium divaricatum* L.Decne

Introduction

Medicinal plant *Thyponium divaricatum* L.Decne (Araceae) have known as rodent tuber that have 30 cm height, whitish tuber, triangular leaves, and unique yellowish flower from the root. Some studies showed the cytotoxic activity of the plant against cancer cells. Several hexane and dichloromethane extracts could inhibit the growth of NCL-H23 non small cell lung carcinoma cell line significantly with IC_{50} < 15 μg/ml (Lay *et. al.*, 2008). Ethanol extracts could against T47D cells with IC_{50} about 632 μg/ml (Nurrochmad *et. al.*, 2011). Hexane extracts of *T. flagelliforme* against P388 murine leukaemia cells and showed IC_{50} 15 μg/ml (Choo *et. al.*, 2001). The active metabolites were mutualism relation between the plants and the microorganisms in it called endophytic (Pimentel *et. al.*, 2011). Endophytic spend all or part their lifecycle colonizing in healthy tissue of hosts and have no apparent disease symptoms and produce bioactive metabolites of pharmaceutical importance (Khan *et. al.*, 2012). Endophytic fungi are mostly unexplored microorganisms and a few studies showed them as sources of pharmaceutical compounds (Orlandelli *et. al.*, 2012). There is no study about endophytic fungi from *T. divaricatum* L. Decne yet. Therefore, the current study was conducted to isolate, and investigate endophytic fungi produced cytotoxic compounds against T47D cells.

Results and discussion

A total of 14 endophtic fungi were isolated from *T.divaricatum* Lodd and fermented in Potato Dextrose Broth for 21days at room temperature. Each of the harvest filtrate of fermentation were extracted with n-Hexan, methylen chloride, ethyl acetate, and methanol. All extracts were tested against T47D cells and value IC_{50} of fungi were described in Table 1.

Table 1 IC_{50} of extracts of endophytic fungi against T47D cells

No	Isolates	IC_{50} of extracts (μg/mL)			
		n-Hexan	Methylen Chloride	Ethyl acetate	methanol
1.	Tf.1F	>100	**10.20**	>100	>100
2.	Tf.2F	>100	>100	>100	>100
3.	Tf.3F	>100	>100	>100	>100
4.	Tf.4F	>100	**10.10**	**23.40**	>100
5.	Tf.5F	>100	>100	>100	>100
6.	Tf.6F	>100	**11.50**	**7.90**	>100
7.	Tf.7F	>100	>100	>100	>100
8.	Tf.8F	>100	**58.80**	**32.00**	>100
9.	Tf.9F	>100	>100	>100	>100
10.	Tf.10F	>100	**58.70**	**52.90**	>100
11.	Tf.11F	>100	>100	**53.20**	>100
12.	Tf.12F	>100	>100	>100	>100
13.	Tf.13F	>100	>100	>100	>100
14.	Tf.14F	>100	>100	>100	>100

The methylene chloride and ethyl acetate extracts of endophytic fungi Tf.1F, Tf.4F, Tf.8F, Tf.10F and Tf.11F could against T47D cells with IC_{50} were about 10.10-58.80 µg/mL. The cytotoxic activity with IC_{50} < 50µg/mL was revealed by methylene extracts from endophytic fungi Tf.1F, Tf.4F and ethyl acetate from endophytic fungi Tf.4F, Tf.8F. It showed that cytotoxic compounds from endophytic fungi were semipolar because they were extracted with methylene chloride and ethyl acetate. The endophytic fungi isolated from Malaysia medicinal plants also showed the cytotoxic compounds against a number cell line from ethyl acetate extracts (Hazalin *et. al.*, 2009). Commonly, cytotoxic metabolites of endophytic were extracted with ethyl acetate. But, this current study showed cytotoxic metabolites could be extracted with methylene chloride. Both methylene chloride and ethyl acetate had antiproliferative and apoptosis activity which needed more study.

In this study, we had reliationship between endophytic and *T.divaricatum* L.Decne. The previous study showed the semipolar extracts of *T.divaricatum* L.Decne had cytotoxic activity so did endophytic (Choo *et. al.*, 2001; Lay *et. al.*, 2008; Nurrochmad *et. al.* 2011). Endophytic could produce bioactive metabolites like its host (Khan *et. al.*, 2012). Several endophytic fungi could not produce cytotoxic metabolites against T47D cells, but the metabolites could have cytotoxic activity against another cell lines and it still needed further study.

The active extracts were conducted to have screening phytochemical. Endophytic Tf.1F, Tf.4f, Tf.8F and Tf.10F extracts had alkaloids, but endophytic Tf.11F extracts had tannin in its cytotoxic compounds as described in Table 2.

Table 2 Screening phytochemical of active extracts of endophytic

No.	Isolates	Phytochemical compounds	
		Methylene chloride extracts	Ethyl acetate extracts
1.	Tf.1F	Alkaloids	Alkaloids
2.	Tf.4F	Alkaloids	Alkaloids
3.	Tf.8F	Alkaloids	Alkaloids
4.	Tf.10F	Alkaloids	Alkaloids
5.	Tf.11F	Alkaloids + tannin	Alkaloids + tannin

Both alkaloids and tannin had cytotoxic activity. Alkaloids could neutralize toxin in body and do antiproliferative cell lines. Tannin had anticancer and apoptosis activity against cell lines. Alkaloids of endophytic *Fusarium incarnatum* had cytotoxic activity against HUVEC, K-562 and HeLa human cell lines (Ding *et. al.*, 2012). *Mycoleptodiscus* sp F0194, an endophytic fungus produced Mycoleptodiscin A and B alkaloids which effective against cancer cell lines (Ortega *et. al.*, 2013).Chaetominine, cytotoxic alkaloids was produced by endophytic fungus Chaetomium sp IFB E-015 and against the human leukemia K562 and colon cancer SW1116 cell lines (Jiao *et. al.*, 2006). The hydrolysable tannin extracted from *Rhizophora apiculata* barks could against HepG2 cancer cells (Hong *et. al.*, 2011). It showed that the active extracts of endophytic fungi from *T.divaricatum* L.Decne. contained alkaloids and tannin could have antiproliferative and apoptosis activity which need to be conducted in further research.

Experimentals

General

Isolation of endophytic fungi was used Laminar Air Flow (Aneka), and incubator 5% CO_2 (NAPCO). Fungi fermentation used shaker (Braun). Analyze of cytotoxic activivity used ELISA Reader (Labsystem). Fungi was isolated and cultivated in PDA (Potato Dextrose Agar), CMM (Corn Malt Media) and Potato Dextrose Broth (PDB) from HiMedia. The invitro assay used the reagents dimethylsulphoxide (DMSO) 100% for dissolving samples, Dulbecco's Modified Eagle's Medium

(DMEM) containing L-glutamine supplemented with 10% v/v foetal bovine serum, sodium bicarbonate, 100 µg/ml streptomycin and 100 U/ml penicillin for cultivating T47D cells, Phosphate Buffer Saline (PBS), Trypsine, Cisplastin, trichloroacetic acid (TCA), Sulphorodamine B, Acetic acid, Tris base (pH.10) from Merck.

Plant Material

The fresh *T. divaricatum* L.Decne were collected from Research Institute of Medicinal Plants and Aromatics (BALITRO), Bogor. The plants were stored at 4^{o}C until been used. The plants were identified by BALITRO, Bogor.

Isolation of endophytic fungi

Endophytic fungi were isolated from leaves, roots and branches of *T. divaricatum* L.Decne. The complete fresh plants were washed under running tap water for 10 minutes. For sterilized surface leaves, roots and branches, they were cut into 1 cm length segments. Sample segments were immersed in etanol 70% for 1 minute, sodium hypochloride 5% for 5 minutes, etanol 70% for 30 seconds and water for 30 seconds. The sterilized segments were put into petridish containing CMM and PDA.

Extraction of bioactive compound

Each of pure cultures was recultivated on PDA plates at 25^{o}C for 7-14 days. Three pieces (0.5 X 0.5 cm) of mycelia agar plugs were inoculated into 500 ml Erlenmeyer flaks containing 150 ml PDB and incubated at room temperature on shaker 150 rpm for 20 days. Each of harvest cultures was desperate to have filtrate and biomass. Filtrate was extrated with MTC, ethylacetat, n-Hexan, butanol, and methanol. Biomass were dried in oven and then extracted with methanol. All the solvent phase of filtrate and biomass were evaporated using rotary vaccum evaporator at 40^{o}C. The crude extracts were analyzed for cytotoxic compounds against T47D cells (Hazalin *et. al.*, 2009).

SRB assay

For cytotoxic activity, each of fungi extracts were dissolved in 100 % DMSO to have stock solutions at 4mg/ml concentration. Final concentration at DMSO 0.5% were achieved by diluting the stock with medium (Ding *et. al.*, 2012). The cells were maintenanced in DMEM containing L-glutamine supplemented with 10% v/v fetal bovine serum, sodium bicarbonate, 100 µg/ml streptomycin and 100 U/ml penicillin at 37^{o}C in a incubator of 5% CO_2. The T47D cells had 80% confluence after 3 days incubation. The cells were washed 1 times with 20 ml PBS, added 1 ml Trypsin solution and incubated for 5 min. Then, fresh medium was added into the cell container. In cytotoxicity assay, 190 µl cell solution was mixed with 10 µl test sample solution (in 10% DMSO) / well and incubated at 37^{o}C for 3 days. It used cisplatin as control. The cells were fixed by adding 50 µl TCA), incubated at 4^{o}C for 30 minutes, washed with tap water 4 times and air dried plates until no standing moisture was visible. Plates containing wells were stained with 100 µl SRB in 1% acetic acid at room temperature for 30 minutes. Then, the plates were rinsed off unbound dye with 1% acetic acid for 4 times and air dried. Amount of 100 µl 10mM Tris base (pH.10) was added into each of the wells to solubilize dye on a gyratory shaker for 5 minutes (Ortega *et. al.*, 2013). The optical density (OD) was measured at 515 nm using an ELISA plate reader and determined the ED_{50} value. Data were calculated as percentage viability using the following formula:

$$\text{Inibition (\%)} = \frac{OD\ (cells+sample) - OD\ (0\ day)}{OD\ (cells+10\%\ DMSO) - OD\ (0\ day)} \text{ X } 100\%$$

IC_{50} values were calculated from Prism program obtained by plotting precentage of survival versus concentrations, interpolated by cubic spine. According to National Cancer Institute guidelines that extracts with IC_{50} value 20-30 µg/ml were considered active (Patel *et. al.*, 2009).

Phytochemical screening

Phytochemical screening were carried out for all active extracts as per standard methods (Tiwari *et. al.*, 2011).

a. Detection of alkaloids: extracts were dissolved in dilute Hydrochloric acid and filtered. Filtrates were treated with Dragendroff's reagents. Red presipitation indicated the presence of alklaoids.
b. Detection of saponin: extracts were diluted with 20 ml dH_2O and shaken for 15 minutes. Formation of 1 cm of foam indicated the presence of saponin.
c. Detection of phenols: extracts were of treated with 3-4 drops of ferric chloride solution. Bluish black colour formation indicated the presence of phenols.
d. Detection of tannin: extracts were added 1% gelation containing sodium chloride. White presipitation indicated the presence of tannin.
e. Detection of flavonoids: extracts were treated with 3-4 drops sodium hydroxide solution. The intense yellow colour which becomes colourless on addition of dilute acid indicated the presence of flavonoids.

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References

Choo, C.Y., Chan, K.L., Tayeka, K, Itokawa H. The cytotoxicity and chemical constituents of the hexane fraction of *Thyponium flagelliforme* (Araceae). *Journal of Ethnopharmacology* 77(1): 129-131, 2001.

Ding, L., Dahse, H.M., Herwech, C. Cytotoxic alkaloids from Fusarium incarnatum associated with the mangrove tree *Aegiceras corniculatum*. *Journal of Natural Products* 75(4): 617-621, 2012.

Hazalin, N.A.M.N., Kalavathy, R., Siong, M.L., Ibtisam A.W., Anthony, L.J.C., Abu, B.A.M. Cytotoxic and antibacterial activities of endophytic fungi isolated from plants at the National Park, Pahang, Malaysia. *BMC Complementary and Alternative Medicine* 9(46): 1-5, 2009.

Hong, L., Darah, I., Jain, K. Assessment of Invivo and Invitro Cytotoxic Activity of Hydrolysable Tannin Extracted from *Rhizophora apiculata* barks. *World Journal of Microbiology and Biotechnology* 27(11): 2737-2740, 2011.

Jiao, R.H., Shu, X., Jun, Y.L., Hui, M.G., Hui, D., Chen, X., Hai, L.Z., Ren, X.T. Chaetominine, a Cytotoxic Alkaloid Produced by Endophytic Chaetomium sp. IFB-E015. Organic Letters 8(25): 5709-5712, 2006.

Khan, A.L., Hamayun, M., Hussai, J., Kang, S., Lee, I. The Newly Isolated Endophytic Fungus *Paraconiothyrium* sp.LK1 Produces Ascotoxin. *Molecules* 17: 1103-1112, 2012.

Lay, C., Mas, R.H.M.H., Nair, N.K., Majid, M.I.A., Mansor, S.M., Navaratnam, V. *Thyponium flagelliforme* inhibits cancer cell growth in vitro and induces apoptosis: An evaluation bioactivity guided approach. *Journal of Ethnopharmacology* 118: 14-20, 2008.

Nurrochmad, A., Likitaningsih, E., Meiyanto, E. Anticancer activity of Rodent tuber (*Thyponium flagelliforme* (Iodd). Blume on human breast cancer t47d cells. *International Journal of Phytomedicine* 3: 138-146, 2011.

Orlandelli, R.C., Alberto, R.N., Filho, C.J.R., Phampile, J.A. Diversity of endophytic fungal community associated with *Piper hispidum* (Piperaceae) leaves. *Genetics and Molecular Research* 11(2): 1575-1585, 2012.

Ortega, H.E., Graupner, P.R., Asai, Y., Tendyke, K., Qiu, D., Shen, Y.Y., Rios, N., Arnold, A.E., Coley, P.D., Kursar, T.A., Gerwick, W.H., Cubilla-Rios L. Mycoleptodiscins A and B, Cytotoxic Alkaloids from the Endophytic Fungus *Mycoleptodiscus* sp. F0194. *Journal of Natural Products* 7(7): 123-128, 2013.

Patel, S., Nirav, G., Ashok, S., Anand, S., In Vitro Cytotoxic Activity of *Solanum nigrum* Extract Against Hela Cell Line and Vero Cell Line. *International Journal of Pharmacy and Pharmaceutical Sciences* 1(1): 38-46, 2009.

Pimentel, M.R., Gustavo, M., Ana, P.D., Mario, R.M.J., Glaucia, M.P. The Use of Endophytes to Obtain Bioactive Compounds and Their Application in Biotransformation Process. *Biotechnology Research International*: 1-11, 2011.

Tiwari, P., Bimlesh, K., Mandeep, K., Gurpreet, K., Harleen, K. Phytochemical Screening and Extraction: A Review. *Internationale Pharmaceutica Sciencia* 1(1): 98-106, 2011.

Optimization of Sucrose Concentration on Somatic Embryos Formation and Maturation of Pasak Bumi (*Eurycoma longifolia* Jack.)

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ABSTRACT - Pasak bumi is widely used for herbal medicine. Its contain many secondary metabolites for various uses. Due to many uses of pasak bumi, overexploitation can causes threaten of pasak bumi in nature. Embryogenesis somatic is one of methods to regenerate plant. Sucrose concentration was known to improve somatic embryo development. This research has been done to determine sucrose concentration in liquid and solid medium for somatic embryo formation and maturation. Embryogenic suspension was transferred to treatment medium. The optimization in liquid medium contained 2 ppm BAP, 1 ppm 2,4-D, and various sucrose concentration (0%, 1%, 2%, 3%, 4%, or 5%), while in solid medium used 2%, 3%, 4%, or 5% sucrose. In liquid medium, the highest somatic embryos was produced in medium containing 3% sucrose (45 globulars/5mL and 14 hearts/5mL) in 8 week. Torpedo could be formed in this treatment, but other treatment failed to produce torpedo stage. In solid medium, the highest somatic embryos was produced in medium containing 2% sucrose (13 globulars and 2 hearts). Based on results, it can be concluded that 3% sucrose in liquid medium was the best medium composition for somatic embryo formation and development.

Keywords: induction, maturation, pasak bumi, sucrose

FULL PAPER ACCEPTED by HAYATI JOURNAL

The Effect of Islet Langerhans Transplantation on Blood Glucose Level of Alloxan Induced Diabetic Rats (*Rattus norvegicus*, Wistar)

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ABSTRACT – Diabetes mellitus is caused by insulin deficiency or insulin resistance and leads to hyperglycemia (blood glucose level (BGL) ≥ 200 MG/dL). Diabetes mellitus affects many people around the world and still can not be cured. One of the methods being developed is stem cell therapies derived from the islets of Langerhans. Although transplantation of islets of Langerhans has been proven to reduce BGL, the amount islets transplated and the method of transplantation is still uncertain. The aims of this srtudy were to transplant normal islets of Langerhans to diabetic rats and analyze the effects of the transplantion on BGL. Diabetic rats were induced with a single dose of alloxan 150 mg/kg body weight administrated by intraperitonial injection. Diabetic rats were divided into three groups: P300, P800, and diabetic control (KD) that were transplanted with 300 islets, 800 islets and solvent respectively. Normal rats were also used as normal control (KN) and injected with solvent. BGL of the entire group were measured every three days for 15 days and analyzed statistically using T-test. On day 16 after transplantion, oral glucose tolerance test was performed. The histology of normal pancreas were observed. Group P300 showed the decrease of BGL, whereas group P800 showed the increase of BGL. Histological results showed that transplantation changed the structure of the pancreas. It can be concluded that transplantion of 300 islets could decrease BGL of diabetic rats, whereas transplantation of 800 islets increased BGL diabetic rats.

Keywords: diabetes mellitus, blood glucoce level, islet langerhans, pancreatic stem cell, transplantation

FULL PAPER ACCEPTED by HAYATI JOURNAL

Antibacterial, Antifungal and Anticancer Activity of Five Strains of Soil Microorganism Isolated from Tangkuban Perahu Mountain by Fermentation Process

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ABSTRACT - Soil microorganisms were isolated from the Tangkuban Perahu mountain. Five strains were investigated in this study which are designated TP1, TP2, TP3, TP4 and TP5. Three of them were identified to be actinomycetes and two of them were fungi. Morphological, biochemical and molecular identification was conducted for all five strains. These isolate were shown to be closely related to *Nocardia sp*. YIM 65630 (90%), *Streptomyces galbus* (99%), *Aspergillus unguis* (86%), *Paecilomyces marquandii* (100%) and *Nocardia niigatensis* (95%) respectively. Production of antibacterial, antifungal and anticancer metabolites was done by fermentation process. Screening for bioactivity of five isolates was done by testing the fermentation broth against bacteria, pathogenic fungi and breast cancer T47D cell line. Strain TP2 showed the best bioactivity and further activities were conducted to isolate and purify the metabolite by solvent extraction. Antibacterial, antifungal and anticancer bioactivity were tesyed from the ethyl acetate extract of strain TP2 by diffusion , agar, microdillution and MTT methods. The extract was shown to be active against *Methicillin Resistance Sthapylococcus Aureus* (MRSA), *Methicillin Sensitive Sthapylococcus Aureus* (MSSA), *Methicillin Resistance Coagulase Negative Sthapylococcus* (MRCNS) , *Vancomycin Resistance Enterococcus* (VRE), *Escherichia. coli*, *Microsforum gypseum* with MIC (minimum inhibitory concentration) (µg/ml) and inhibition diameter (mm) respectively: 150, 35; 150, 30; 300, 35; 300,35; 300, 29; 4.7, 36 and IC_{50} of T47D cell line was 457 µg/ml.

Keywords: antibacterial, antifungal and anticancer activities, identification, fermentation, ethyl acetate extract.

FULL PAPER WITHDRAWED by AUTHOR

Preliminary Study of Anticancer Activity from Brown Algae (Phaeophyta) Extracts

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ABSTRACT - The pharmaceutical industry continues to show interest in the development of plant-based drug. The secondary metabolites of the brown algae (Phaeophyta) have potent activity in the brine shrimp cytotoxicity assay. Cytotoxic activity by plant material is considered to be an indicative of the presence of anticancer compound. In this study, n-hexane and ethyl acetat extracts of five brown algae were screened for cytoxic activity in brine shrimp. This study found that n -hexane extract of *S.myriocystum* showed the highest toxicity in the brine shrimp assay (LC_{50}= 273.28 µg/ml). This extract tested positive for triterpenoids and steroids.

Keywords: *brown algae, Phaeophyta, brine shrimp lethality test, anticancer*

FULL PAPER WITHDRAWED by AUTHOR

Expression and Purification of Fusion Gene HBcAg-HBsAg in *Escherichia coli* Recombinant

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ABSTRACT - Hepatitis B Virus (HBV) is a common infectious disease with an estimated 450 million chronic HBV carriers worldwide. Patients with chronic hepatitis B may develop hepatocellular carcinoma and liver cirrhosis. HBV vaccine is available however there is room for the development of an effective and cheaper vaccine. Hepatitis B surface antigen (HBsAg) and hepatitis B core antigen (HBcAg) play an important role in controlling the infection. CD4+T cells for both antigens induce humoral and cytotoxic T lymphocyte (CTL) immune response. HBcAg can form virus-like particle which can also be used to insert foreign epitope.Tthe purposes of this research is to clone and express of HBcAg-HBsAg fusion gene in *Escherichia coli*. HBcAg-HBsAg gene was cloned into pET32b expression vector in order to simplify the purification process. The DNA construct was expressed in *E.coli* BL21 (DE3). The recombinant protein was purified using HiTrap™ Chelating HP 1 ml column and 0.25 M imidazole as elution for the protein. Expression analysis in *E.coli* by SDS PAGE and purification using Histidine-tag showed that this protein is expressed as inclusion bodies and indicates that the protein has a size approximately 25 kDa.

Keywords: Fusion gene HBcAg-HBsAg, HBV, inclusion bodies, protein purification, SDS-PAGE.

FULL PAPER WITHDRAWED by AUTHOR

Antidiabetic and Antidiarrheal Activity of Ant Plants (*Myrmecodia pendens* Merr & Perry)

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ABSTRACT - The high concentrations of flavonoids and tannins in Ant plants (*Myecodia pendens* Merr & Perry) makes them candidates as a source of antidiabetic and antidiarrheal drugs. Here we show that decocta derived from ant plants have antidiabetic and antidiarrheal activity.. Plants were extracted through decoction, subjected to phytochemical screening, and antidiabetic and antidiarrheal activity tested in Swiss-Webster mice. The results showed that the ant plants contain tannins, flavonoids, quinones, and saponins and that a dose of 26 mg decocta/20 g mouse BW had both antidiabetic and antidiarrheal activity..

Keywords: Ant plants, Antidiabetic, Antidiarrheal, Chemical constituens, Decocta

FULL PAPER WITHDRAWED by AUTHOR

The In silico binding interaction of Oseltamivir Derivatives with H7N9 Haemagglutinin and Neuraminidase

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ABSTRACT - The H7N9 virus has caused severe mortality in China. Therefore, it is necessary to develop an effective drug to tackle its infection. The in silico utilization of Oseltamivir Derivatives has successfully showed binding interaction with Haemagglutinin and Neuraminidase of H7N9. In this end, those lead compounds could be developed as drug candidates.

Keywords: drug candidates, H7N9, haemagglutinin, in silico, neuraminidase, oseltamivir, Virus

The Potency of Propolis as Reactive Oxygen Species Inhibitor in Diabetic Mice

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ABSTRACT - Diabetes mellitus (DM) is a chronic disease characterized by hyperglycemia for a long time. Hyperglycemia is proven increasing oxidative stress due to the production of reactive oxygen species (ROS) that exceeds the ability of the natural antioxidant defenses, causing deficiency and insulin resistance. In this study, the effect of propolis on ROS was observed. Fivety five (55) male mice (*Mus musculus* SW.) were divided into 5 groups, ie: groups of KN (normal control), P1, P2, P3 and KDM (diabetes control) induced to be diabetes by using alloxan dose 200 mg/kg bw intraperitoneally. Propolis solution 50, 100 and 175 mg/kg bw were given to P1, P2 and P3, while distilled water was given to the KN and KDM by oral gavage for 21 days. Density of ROS was measured every 7 days, while the measurement of plasma insulin is carried out every 3 days. The results showed that the lowest density of ROS was found in pancreatic of KN group (9.32 ± 1.59 mm²/mg tissue), followed by P3 (44.85 ± 22.55 mm²/mg tissue), P1 (54.41 ± 23.73 mm²/mg tissue), P2 (79.13 ± 38.08 mm²/mg tissue and DM (109.58 ± 20.77 mm² mg tissue). The highest plasma insulin levels was found in KN group (3.50 ± 0.370 µg/mL) followed by P3 (02.44 ± 0.146 µg/mL), P1 (2.44 ± 0.453 µg/mL) and P2 (2.22 ± 0.333 µg/mL). KDM has the lowest plasma insulin levels, 1.41 ± 0.286 µg/mL. From this study, we concluded that propolis can decrease ROS density that results and causing an increase of plasma insulin levels.

Keywords: alloxan, diabetes mellitus, hyperglycemia, oxidative stress, propolis reactive oxygen species.

FULL PAPER ACCEPTED by ITB JOURNAL

Primary Monkey Trachea Cell Culture Express the Substantial Components for Influenza Virus Internalisation

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Abstract. A number of mammalian cells have been used as substrates for influenza virus. We presume that they have sufficient molecules that are important for influenza virus internalisation. Influenza virus needs membrane protein contained Sia(α2,6) Gal as the receptor. The α2,6 sialyltransferase (SIAT1) is required for Sia(α2,6) Gal glicosylation. On the other hand, light clathrin A (LCA), light clatrin B (LCB) and heavy clathrin (HC) are the main molecules needed for influenza virus endocytosis. The aim of our present study is to find the expression of sialyltransferase and clathrin observed in primary monkey trachea cell culture in the level of (i) RNA by using RT-PCR and (ii) protein level by using confocal microscope. Since MDCK cell culture is the most widely used in influenza virus research , we compared the components of primary monkey trachea cell culture to MDCK cells. The results proved that primary monkey trachea cell culture expressed both sialyltransferase and clathrin proteins. The expressions of SIAT1and HC in primary monkey trachea cells were significantly higher compared to MDCK cells. Otherwise, the expressions of LCA and LCB protein in monkey trachea cells were not significantly different to MDCK cells. This results support as appropriate data that primary monkey trachea cell cultures could be used as a candidate of human influenza virus substrate.

Keywords: *Clathrin, Foetal Primary Monkey Trachea Cell, Sialyltransferase.*

FULL PAPER WITHDRAWED by AUTHOR

Histopathological Changes in Rat's Substantia Nigra pars Compacta and Pyramidal Tract Exposed to Crude Extract from *Derris elliptica* Benth Roots

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Abstract. *Derris elliptica* Benth has long been used as a biopesticides and is considered more secure and environmentally friendly than synthetic pesticides. However, *Derris* contained rotenone. One well known neurotoxin that affect multiple brainstem neuron cause like in Parkinson's syndrome.This research aimed to observe the effects of subchronic exposure of crude extract from *Derris elliptica* Benth roots on nerve tissue in rat's brainstem mainly in substantial nigra pars compacta (SNc) and pyramidal tract. Male and female rats (8-10 weeks, 140-200 g) were divided into three groups. *Derris* root crude extract groups were given 15.84, 25.11, 39.80 or 63.09 mg/kg bw daily for 28 days. Negative and positive controls groups, each were given VCO and rotenone (2.5 mg/kg bw) with the same duration. These substances were administered via intraperitoneal injection. Forty eight hours after the last injection, the rats were perfused via cardiac. The midbrain and medulla oblongata were removed, and then processed for histological observation. Histopathological changes were found in this study are neuronal hypertrophy and glia cells hyperplasia. These histopathological features were calculated from coronal sections of midbrain and from transverse sections of medulla oblongata. Neuronal hypertrophy–number increased in a dose-dependent manner with significant differences to negative controls ($p<0.05$). Neuronal hypertrophy number at the highest *Derris* dose (63.09 mg/kg bw) and positive control in SNc and pyramidal tract showed significant difference to negative controls. Glia cells hyperplasia number tend to increase but showed no significant difference to negative controls. Based on the results, crude extract from *Derris elliptica* roots at dose 63.09 mg/kg bw caused nerves tissue damage on SNc and pyramidal tract.

Keywords: *crude extract, hyperplasia, hypertrophy, Derris elliptica* Benth roots, *glial cells, pyramidal tract, substantia nigra pars compacta, rat*

FULL PAPER ACCEPTED by HAYATI JOURNAL

Subchronic Exposure Effect of Crude Extract from *Derris elliptica* Benth Roots on Rat's Balance and Motor Coordination

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Abstract. In Indonesia *Derris elliptica* Benth, has been used as natural pesticide that considered more secure than synthetic pesticide. However, *Derris* contains rotenoids active compounds such as rotenone that causes Parkinson's syndrome characterized by impairment in motor function. The purpose of this study was to observe the effect of *Derris* root subchronic exposure on rat's balance and motor coordination using rotarod test and challenging beam traversal test. Male and female rats (8-10 weeks, 140-200 g) were divided into three groups. *Derris* root crude extract groups were given 15.84, 25.11, 39.80 or 63.09 mg/kg bw daily for 28 days. Negative and positive controls groups, each were given VCO and rotenone (2.5 mg/kg bw) with the same duration. Each substance injected intraperitoneally once per day for 28 days. Twenty-four hours after the last injection, rotarod test and challenging beam traversal test were performed. The ability of *Derris*-treated rats to maintain their balance and motoric coordination increased with the rotarod acceleration speed, but only significantly different ($p<0.05$) from the vehicle control group at the highest dose. On the challenging beam traversal test, treated rat's balance and motor skill disorder performance increased in a dose-dependent manner with significant differences to vehicle control ($p<0,05$) at two highest doses. It can be concluded that the rats treated with crude extract from *Derris* roots at dose 39.80 and 63.09 mg/kg showed impairments in balance and motor coordination .

Keywords : *challenging beam traversal test, motor coordination, rotarod test, crude extract, Derris elliptica Benth Roots*

FULL PAPER WITHDRAWED by AUTHOR

The asymetry of Rat's Forelimb Spontaneous Movement Following Administration of Crude Extract from *Deriis elliptica* Benth Roots

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Abstract. Biopesticide is a result of the utilization of plant extracts as an alternative to deal with pest's problem to chemical pesticides, which can cause health problems and pollution. *Derris elliptica* Benth. is often used as biopesticide because the containment of rotenon as its active compound, especially in the roots part. Parkinson's syndrome, which one of them is the disruption of forelimbs' motor activity. Therefore, *Derris*, which often used as a biopesticide is feared could cause the same disruptions. The purpose of this study is to observe the subchronic effect of crude extract from *Derri*s root on the asymetry of rat's forelimb spontaneous movement. This research used four doses of crude extract from *Derri*s root (15.84; 25.11; 39.80; 63.09 mg/kg bw), VCO (as solvent control) and rotenone 2.50 mg/kg body weight (as positive control). Each substances were injected to males and females rats (8-10 weeks, 140-200 g) intraperitoneally once per day for 28 days. Cylinder test and reaching test tests performed 24 hours after the last injection. Cylinder test results shows asymmetry between the movement of right and left forelimbs, both in male and female rats. The numbers of movement of left forelimb always lower than the right forelimb. The asymetry of rat's forelimb spontaneous movement increased in a dose-dependent manner, although not significantly different. Meanwhile, the ability of both male and female rat's forelimbs in reaching an object decline in a dose-dependent manner, although not significantly different. Based on the results, it can be conclude that crude extract from *Derris* root has the potential to disrupt rat's forelimb spontaneous movement.

Keywords: *forelimb, cylinder test, crude extract, Derris elliptica root, reaching test, rats*

FULL PAPER WITHDRAWED by AUTHOR

Optimization of Fermentation Process of Pummelo (*Citrus grandis*) Juice with Variation of Sucrose Levels Using *Sachharomyces cereviciae* to Improve Total Phenolic Content as Antioxidants.

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ABSTRACT - Demand for natural antioxidants has increased due to the risk of toxic effects and other side effects caused by application of synthetic antioxidants. Jeruk bali (*Citrus grandis*) is known to have good antioxidant potential. Antioxidant content in jeruk bali is identical to the phenolic compounds they have. It is known that the content of total phenolic compounds in the ethanol fermentation product from jeruk bali is higher than in the fresh juice. The optimization study was conducted on juruk bali pulp juice using *Saccharomyces cerevisae* with the 12 hours inoculum and incubated for 72 hours at 25^{o}C with variation of sucrose concentration of 13^{0}, 15^{0}, and 17^{0} Brix. During the fermentation process was measured three types of growth parameter from *S. cerevisiae*, those are pH, biomass, dan changes in level of sugar (^{o}Brix). Each 24-hour alkohol content was measured using ethanol titration method, the content of total phenolic compounds was tested with the Folin-Ciocalteu colorimetric method dan antioxidant activity is converted into a percent (%) of DPPH radical-scavenging activity. The highest specific growth rate of *Saccharomyces cerevisiae* in medium consists of jeruk bali pulp juice with sucrose level ranging from 13^{o} Brix, 15^{o} Brix, and 17^{o} Brix is 0.116 jam^{-1}, 0.113 jam^{-1} ; dan 0.115 jam^{-1} in 0 to 8 hours of fermentation time. From two variations of the sucrose levels of 15^{o} and 17^{o} Brix, content of total phenolic compound reach the optimum concentration at 48 hours of fermentation time, that is 69.9 µg/mL GAE and 67 µg/mL GAE with the rate of increase in total phenolic content measured at 0,077 µg/mL GAE hour^{-1} and 0,026 µg/mL GAE hour^{-1} in 0 to 48 hours of fermentation time, while the highest content of total phenolic compounds in the medium consists of jeruk bali (*Citrus grandis*) juice with 13^{o} Brix sucrose levels was occured at 72 hours of fermentation time, that is 66.7 µg/mL GAE with with the rate of increase in total phenolic content measured at 0.034 µg/mL GAE hour^{-1} in 0 to 72 hours of fermentation time. From the resuls of the study concluded that fermented pulp juice of jeruk bali (*Citrus grandis*) using *Saccharomyces cerevisiae* culture with sucrose level of 15^{o} brix can increase the content of total phenolic compounds at its optimum.

Keywords: *Citrus grandis*, sucrose, *S. cerevisiae*, total phenolic compounds, antioxidants

FULL PAPER WITHDRAWED by AUTHOR

Medicinal Compounds Production by *Vetiveria zizanioides* Root Cultures

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ABSTRACT - *Vetiveria zizanioides* has been widely used traditionally for centuries to produce vetiver oil as an aroma therapy. Other researches of secondary metabolite in roots showed compounds that served as medicinal compounds. The advantages cause increasing demand of roots. Therefore, alternative technologies are required to produce *V. zizaniodes* roots, known as root culture. The objectives of this research to optimize growth of root culture which produce medicinal compounds for pharmaceutical industry and traditional herbs. The research showed that the root culture which derived from crown explants produced the best growth, consist of three primary lateral roots with an average length of 5.20 cm. They also produced 40 secondary lateral roots with an average length of 0.48 cm. Extract of root culture also showed compound related to medicinal properties. They consist of 6.34% 1.2-Benzenedicarboxylicacid diisooctylester, 0.19% 1-Methyl-4-(2 -[(4methylphenyl) sulfonyl]ethyl) piperazine and 2.16% 2-(diethylamino)-2-oxo-5,5-dimethyl-1,3,2-oxazaphosphorinane. The results confirmed that *V. zizanioides* root culture have a potential to be use as alternatives technology for secondary metabolite production the pharmaticeutical and traditional herbs industry.

Keywords: Medicinal Compounds, Root culture, *Vetiveria zizanioides.*

FULL PAPER WITHDRAWED by AUTHOR

Construction and Expression of a Synthetic Gene Encoding protein NS1 from Dengue Virus-3 as A Target Molecule for Development of Diagnostic Kits

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ABSTRACT – Dengue virus (DENV) carried by the *Aedes aegypti* mosquito causes a spectrum of ilnesses including dengue fever, dengue hemorrhagic fever, dengue shock syndrome. According to the World Health Organization (WHO), 2,5 billion people who are living in the tropic and sub tropic areas are at risk for epidemic transmission of this virus and 50 million of them are annualky infected with mortatlity rate of 2,5%. To more fully characterize the illness and reduce its mortality rate, a rapid and accurate detection of dengue virus during febrile stage of the disease is essentially needed. This research aims to construct and express a gene encoding NS-1DENV3 protein, which is an important biomarker for early diagnosis of the disease, heterogenously in *Escherichia coli*. The nucleotide sequence of the gene is generated from the NCBI database with optimized codons for expression in *E. coli*. The gene subsequently was inserted to an expression with Glutathione S-transferase (GST), producing pGS21a-DENV3. As determined by SDS-PAGE analysis, the expressed protein was a band with size of ~70 kDa. This result was in accordance with size of the fusion protein between GST (~30kDa) and NS1-DENV3 (~40kDa).

Keywords: biomarker, dengue disease, dengue virus, diagnostic kit, protein NS1

FULL PAPER WITHDRAWED by AUTHOR

Isolation and Construction of 3-Hydroxy-3-Methylglutaryl-coenzyme a Reductase 1 Gene (hmgr1) from *Andrographis paniculata* (Burm.f) Wallich. ex Nees

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ABSTRACT – Andrographolide, is the major bioactive compound in *Andrographis paniculata*. It is known to be useful as anti-oxidant, anti-microbial and anti-cancer. Hmg-CoA reductase (3-hydroxy-3-methylglutaryl-coenzyme A reductase) which is one of the enzymes involved in the mevalonate pathway may affect the presence of andrographolide in *A. paniculata* plants. Increased expression of hmgr1 genes that encode hmgr1 enzyme may increases andrographolide on *A. paniculata*. On this research has been done isolation and construction of hmgr1 gene on binary vector pBI121. Based on alignment hmgr1 gene from mRNA and genomic DNA *A. paniculata* (GenBank, accesion number AY429658 and AF389879) is known that there is no intron in hmgr1 genes, therefore hmgr1 gene can be isolated from total DNA *A. paniculata* through PCR (Polymerae Chain Reaction) using specific primers. Construction has been done using XbaI for restriction, T4 ligase for ligation, and *E.coli* DH5α competent cell as a cloning agent. The succesful of isolation and construction of hmgr1 gene were known through BLAST anlysisis of the sequencing results. It showed the hmgr1 gene (~1815bp) has been isolated from *A. paniculata*. pBI121 recombinat was expected to be used for stable transfer hmgr1 gene to obtain transgenic *A. paniculata* to produce andrographolide more than the natural plants.

Keywords: *A. paniculata*, 3-hydroxy-3-methylglutaryl-coenzyme A reductase, hmgr1, pBI121

FULL PAPER WITHDRAWED by AUTHOR

The Expression Pattern of GH1 in Normal and Propoxur-induced Abnormality in Zebrafish (*Danio rerio*) Embryos

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ABSTRACT – Growth hormone (GH) is GH/PRL protein superfamily member whose important role in growth, development and metabolism. Specific function of GH in Zebrafish embryo development still on study and gh1 temporal expression is not very clear. Hence, this research aimed to confirm gh1 temporal expressions's and pattern in normal and abnormal Zebrafish embryo in affected of propoxur exposure. Propoxur, a carbamate pesticide, has been known can induce developmental abnormality in Zebrafish embryo. RT-PCR, gel electrophoresys and digitalization method with imageJ were used in this study to observe gh1 expression pattern at 0, 24, 48 and 72 hpf, both in normal and abnormal embryo. Abnormality study were obtained by expossing the Zebrafish embryo to a series of propoxur concentration (1.5, 2.0, 2.5, 3.0, and 3.5 ppm) and observed at 24, 48, and 72 hpf. This study shows that in normal embryos, gh1 can be detcted as early as 0 hpf and continue to increase during embryos development, Propoxur exposed embryos developed some defects such as curve body axis, shorter body length, pericardia and yolk sac edema and blood clothing. gh1 gene expression pattern also changed along with the change of propoxur concentration, suggested that there is some compensation mechanism for growth retardement adnd developmental damages in Zebrafish embryo.

Keywords: GH, gh1 gene expression, propoxur, Zebrafish, embryo abnormalities

FULL PAPER WITHDRAWED by AUTHOR

Mixed Culture Fermentation With *Rhizopus oligosporus* and *Micrococcus luteus* to Enhance Isoflavone Aglycone and Factor 2 Production and Antioxidant Activity of Soybean

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Abstract. In this study, mixed culture fermentation of soybeans under solid state fermentation using *R.oligosporus* and *M.luteus* was performed. *M.luteus* was inoculated at different time of fermentation process, together with *R.oligosporus* at the beginning, and after 6 h, 12 h, 24 h, and 36 h fungal fermentation. The methanolic extracts of soybeans were analyzed using HPLC and subjected to free radical scavenging activity measurement using DPPH method. The results of HPLC analysis showed that inoculating *M.luteus* after 24 h fungal fermentation did increase total isoflavones (daidzein, genistein, factor 2) concentration by 69,69% to highest level. The amount of daidzein, genistein, and factor 2 increased to 739,165 µg (75,74%), 805,855 µg (69,99%), and 91,542 µg (31,22%) per gram of deffated soybean powder respectively after 48 h. *M. luteus* inoculation at 0 h, 6 h, 12 h, 24 h, and 36 h of fermentation process increased the soybean free radical scavenging activity to 64,28%, 65,03%, 77,54%, 57,81%, and 82,55%, respectively. This study shows that inoculation time of *M. luteus* plays an important role in the production of daidzein, genistein, and factor 2 at the end of soybean fermentation and affects free radical scavenging activity of soybean.

Keywords: antioxidant, daidzein, factor 2, genistein, *Micrococcus luteus*, *Rhizopus oligosporus*, soybean

The Influence of Plant Growth Regulators on Callus Induction In Vitro Culture Development of *Rauwolfia serpentina* Benth Ex Kurz

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ABSTRACT - *Rauwolfia serpentina* Benth Ex Kurz has 3 alkaloid group : I. Strength alkaline : serpentine, serpentinin, sarpagine, samatine; II. Yohimbine : ajmaline, ajmalicine, tetraphylline, tetraphyllicine; III. Weak alkaline : reserpin, rescinamine, deserpidine, raunesin, canescine (Sudha, 2003). Plants remain the principle source many used for hypotensif, antipyretic, malaria, tiphus, defense from insect. In addition, it has economic value as export product to many countries in the world such as Japan, Germany, France, Switzerland, United Kingdom. Rauwolfia is difficult to conserve and find. Metabolic engineering approach with culture in vitro such as culture cell, calli. Culture in vitro uses medium with growth regulations of kinetin and NAA. The purposes of the research to determine concentration of kinetin and NAA (1,5 ppm, 2,0 ppm, 2,5 ppm) and kinetin (1,5 ppm; 2,0 ppm; 2,5 ppm). The analysis of growth and development of in vitro culture will be measure to dry weight, fresh weight on 1,2,3,4 week after culture. Research will be conducted in Biotechnology cell and Moleculer at SITH Institute of Technology Bandung from year 2011 until 2012. The materials are explants from leaves of *Rauwolfia serpentina*. Research method consists of I. sterilizatIion of tool and medium; II. preparation; III. culture development. Result of research are: fresh weight callus induction on 1,2,3,4 week after culture has significant (Table 1,2), likewise for dry weight callus induction on 1,2,3,4 week after culture has highly significant (Table 5,6), plants have good growth and development. Plant growth regulation (NAA,Kinetin) had role importantly for plant growth and development.

Key words : *Rauwolfia serpentina*, in vitro culture : callus, NAA,Kinetin.

FULL PAPER WITHDRAWED by AUTHOR

HEALTH - 22

Stable Transformation of Medicinal Plant *Andrographis paniculata* Callus Mediated by *Agrobacterium tumefaciens*

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ABSTRACT- A cell clones with a desired traits such as a high content of a secondary metabolites can be obtained through genetic transformation. In order to obtain a successful genetic transformation in cell culture of *A. paniculata*, stable transformation within generations of the cell culture need to be established. The purpose of this research was to established a stable transformed callus culture of *A. paniculata* containing a transgenes of ß-glucuronidase (*GUS*) and hygromycin phosphotransferase (hpt) genes as reporter gene and selectable marker gene, respevtively. Prior to genetic transformation, leaf disks of *A. paniculata* were pre-cultured on callus induction medium (Murashige and Skoog medium containing 0.5 µM 2,4-D + 0.1 µM BAP) for three days. The leaf disks tissues were infected with *Ag. tumefaciens* LBA4404 which contained binary vector pCAMBIA1304 that carries GUS and hpt genes for 60 minutes followed with co-cultivated in the dark for three days. To examine GUS expression, activity of ß-glucuronidase was assayed on infected leaf disks and transformed callus. This transient expression took 2-3 days. Meanwhile, to select stable transformed callus, the infected leaf disks tissues were grown on callus induction medium contains 20 mg l^{-1} hygromycin as selectable agent for 3 weeks. The results indicated that, 64.44 % of the transformed tissue could grow and develop to callus on that medium.The callus was maintained by subcultured it into fresh callus induction medium at interval 3 weeks for 5 passages. Stable transformation was examined by detection of the ß-glucuronidase (*GUS*) gene by PCR analysis of the DNA callus cuture and by enzymatic assays by measuring ß-glucuronidase (*GUS*) activity within five periods of subculture. ß-glucuronidase (*GUS*) histochemical assay indicated the presence activity of ß- glucuronidase enzyme in transformed callus from subculture 1 to 5. The PCR analysis indicated the presence 976 bp bands on the transformed callus from subcultures 1-5 that confirmed the presence of ß-glucuronidase (*GUS*) gene. Based on the results, it was confirmed that the ß-glucuronidase (*GUS*) were stably integrated into *A. paniculata* genome on induced and subcultured callus from first until the fifth subcultured.

Key words: *Andrographis paniculata, Agrobacterium tumefaciens*, transformed callus, *gusA* gene, *hpt* gene

FULL PAPER WITHDRAWED by AUTHOR

POSTER - 01

Microbes as A Source of Enzyme

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ABSTRACT- Microorganism are known as a source of drug discovery and enzymes production. The production of novel metabolites from microorganisms suggests that microbes are very important for research on microbial natural product. One group of microorganisms that have been identified for our further research is the Rhodococcus, which has been proved capable on producing secondary metabolites. *Rhodococcus* also has been confirmed to be a scientifically beneficial system for the production of enzymes. This genus is known for its very diverse and broad spectrum catabolic enzymes and provides a rich source of potential green chemistry. It is a locally isolated strain that capable of degrading xenobiotic and hazardous compounds. This will develop a fundamental understanding mechanism of the enzyme. Phenol hydroxylase is the most important enzyme discover from this microorganism for the degradation of phenol. Phenol is toxic compounds exist in environment due to the activity of chemical, petrol or pharmaceutical industries. Nitrile compound are used extensively in the chemical industry. They are discharged into the environment in industrial waste water, agriculture and chemical. Nitriles are the compound that was contributes to the environmental problem. The possibility of application of nitrilase enzymes in *Rhodococcus* species is now being increasingly recognized and for the example, for the production of a useful acid, and for the streo- and region-specific conversion of nitriles. The nitrilase from Rhodococcus species showed the high yield and high specificity and have considerable potenctial for industrial application for the production of useful compound such as acrylamide, nicotinamide and several vitamins. Meanwhile, it is also interesting to note that the utilization of *Rhodococcus* in degrading cynide by the action of cynide hydratase enzyme is rather promising. Based on enzymes studied, *Rhodococcus* also has the ability to produce intracellular lipase, the fat degrading enzyme that capable to catalyse biodiesel production from waste cooking oil. This is demonstrated that *Rhodocccus* is a versatile bacterium which has a great potential to be used industrially for the production of enzymes.

Key words: *Rhodococcus*, degrading, enzymes, phenol, nitrile, biodiesel

POSTER - 02

Spices and Herbs as a Sources of Nanosizes Antioxidant for Food Quality

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ABSTRACT- Spices and herbs have been the basis of traditional medicines and flavouring food throughout the world for thousands of years and continue to provide new remedies to humankind. There are known for their antioxidant properties, antibacterial, antifungal and have the ability to produce multidimensional flavours in food. The main dietary constituents contributing to medicinal effect are the antioxidant components as well as a wide variety of free radical scavenging molecules such as phenolic compounds, vitamins, alkaloids, and terpenoids. However it is being reported that there has low absorption in the body system and in food due to their larger particle size, complex chemical structure and poor water solubility of the constituent compounds. Several researchs has been proved that particle size reduction can improve solubility and increase the dispersion rate of poorly water –soluble active ingredients. Nanosuspension technology is believed can improve the characteristic of macrostructured of spices and herbs by enhancing their water solubility, bioavailability as well as antioxidant properties which is it helps the active ingredients to disperse and dissolve stably and homogenously

POSTER - 03

Probiotics as The Source of Bioactive Compound with Antimicrobial Activity

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ABSTRACT- Lactic acid bacteria (LAB) is a gram positive, either rod or coccus shape, catalase and an oxidase with negative reaction and produce lactic acid as their end product. These LABs are frequently isolated from fermented foods, diary/poultry products and gastrointestinal tracts ofanimals and humans. Members of LAB are known as probiotic which beneficially affect the host upon ingestion tract health, enhancing the immune system, synthesizing and enhancing the biovailability of nutrients, reducing symptoms of lactose intolerance, decreasing the prevalence of allergy in susceptible individuals and reducing risk of certain cancers. They are capable to produce inhibitory substances such as bacteriocins, lactic acid, hydrogen peroxide, diacetyl, carbon dioxide and low molecular weight antibacterial substances. The effects of a certain probiotic executes depends on its metabolic properties where the molecules presented at its surface or on the components secreted. One important attribute of LABs is their ability to produce antimicrobial compounds that inhibit and control the pathogenic bacteria. In recent years, interest in the compounds has grown substantially due to their potential usefulness as natural substitute for chemical food preservatives in the production of foods with enhanced shelf life and/or safety. The aim of this study was to determine the antibacterial activity of 168 LABs isolates from dairy and non-dairy source (fruits and vegetables) against *Escherichia coli* ATCC 25922 and *Escherichia coli* O157. The antibacterial activity was investigated using well diffusion method. This study revealed the possibility of using bacteriocin as food biopreservative and may help to prevent and treat gastrointestinal infections caused by *E. coli.*

Key words: Lactid acid bacteria, probiotics, antimicrobial

Total Phenolic, Flavonoid, Carotenoid Content in Various Extracts of White Guava Leaves and Correlation with Antioxidant Capacities

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ABSTRACT- Guava **(***Psidium guajava* L.) are well known plant and widely planted in Malaysia and Indonesia. It has many uses such as leaves extract was found to possess antidiarrhea, antimicrobial, hepatoprotective and antioxidant activities. This research aim to determine antioxidant capacity from various extracts of white guava leaves using DPPH assay, IC_{50} of DPPH scavenging activity, total phenolic, flavonoid, carotenoid of each extracts, analyze the correlation between phenolic, flavonoid, carotenoid content and DPPH scavenging capacities. Ethyl acetate extract of of white guava leaves (with density 1% extract 0.89 g/mL) demonstrated the highest DPPH scavenging activity (93.15%), total phenolic 3.02 g GAE/100 g, total flavonoid 10.3 g QE/100 g and total carotenoid 2.32 g BET/100 g. Antioxidant capacity of ethyl acetate extract of white guava leaves 93.15 % was significantly different with ethanolic extract 15.93 % and n-hexane extract 34.75 % ($p<0.05$). IC_{50} of DPPH scavenging capacity of ethyl acetate extract was 6.03 ppm, while ascorbic acid 2.18 ppm. Total flavonoid of ethyl acetate extract had positively high correlation with DPPH scavenging activity, while total phenolic and total carotenoid content had negative correlation with DPPH scavenging activity. Flavonoid was the main contributor in antioxidant activities of ethyl acetate guava leaves extract. Potency of antioxidant of ethyl acetate extract of white guava leaves was one third of ascorbic acid.

Keywords: *antioxidants, DPPH, carotenoid, flavonoid, phenolic, white guava, leaves*

POSTER - 05

In vitro Antioxidant Capacities of Various Extracts of Red Kidney Bean Seeds and Correlation with Total Phenolic, Flavonoid, Carotenoid Content

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ABSTRACT- Cancer and cardiovascular diseases are caused by free radicals. Scavenging free radicals capacity by antioxidant can prevent and cure the diseases. Red kidney bean (*Phaseolus vulgaris* L.) seed that is consumed as food contains some antioxidant compounds, such as phenols and flavonoids. The objectives of this research were 1) to analyze antioxidant activities of various extracts of red kidney bean seeds by DPPH method, IC50, total phenolic, flavonoid, and carotenoid content of each extracts, 2) analyze the correlation between total phenolic, flavonoid, carotenoid content and DPPH scavenging activities. Ethanol extract of red kidney bean seeds (density of 1% extract 0.81 g/mL) showed the highest DPPH scavenging activity (45.33%), with IC50 of DPPH scavenging activity 225.9 ppm, total phenolic 1.50 g GAE/100 g, total flavonoid 1.63 g QE/100 g, total carotenoid 0.28 g BET/100 g. DPPH scavenging activity of ethanol extract of red kidney bean seeds was significantly different with ethyl acetate and n-hexane extract ($p<0.05$). Total phenolic, flavonoid, carotenoid content in ethanol extract of red kidney bean seeds had no significant correlation with DPPH scavenging capacities.. Phenolic, flavonoid, and carotenoid were not the major contributor in antioxidant capacities of red kidney bean seeds.

Keywords: antioxidants capacities, DPPH, carotenoid, flavonoid, phenolic, red kidney bean

APPENDIX I

List of Manuscripts Accepted to be Published at Journal of Mathematical and Fundamental Sciences (ITB Journal)

No	Paper Number
1	Fertilizer 07
2	Fertilizer 08
3	Fiber 02
4	Fiber 04
5	Fiber 05
6	Fiber 08
7	Food 01
8	Food 02
9	Food 06
10	Food 12
11	Food 14
12	Food 17
13	Food 23
14	Fuel 05
15	Fuel 06
16	Fuel 08
17	Fuel 09
18	Fuel 11
19	Health 10

APPENDIX II

List of Manuscripts Accepted to be Published at Hayati Journal of Biosciences

No	Paper Number
1	Feed 07
2	Fertilizer 06
3	Fiber 01
4	Food 03
5	Food 15
6	Food 16
7	Food 20
8	Health 03
9	Health 04
10	Health 05
11	Health 12

APPENDIX III

List of Reviewers

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1.	Dr. sc.agr. Stephan Wirth	Institute for Landscape Biogeochemistry, Germany
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3.	Sun Ji-Quan, PhD.	Peking University, China
4.	Dr. Xu XingJian	Peking University, China
5.	Xing-biao Wang, PhD	Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, China
6.	Dr. Dina Yulia	CSIRO Plant Industry, Australia
7.	Dr. Jean Yves Paul	Queensland University of Technology, Australia
8.	Dr. Sastia Putri	Osaka University, Japan
9.	Prof. Tim Hirst	Gamma Vaccines Pty Ltd, Australia
10.	Dr. Caecilia Sukowati	University of Trieste, Italy
11.	Salim Hiziroglu, Ph.D.	Oklahoma State University, USA
12.	Dr. Hideyuki Takahashi	Kochi University of Technology (KUT), Japan
13.	Prof Shigehiko Suzuki	Shizuoka University, Japan
14.	Dr. Ines Atmosukarto	Lipotek, Australia
15.	Dr. Herry Utomo	Louisiana State University – USA
16.	Dr. Tjandra Anggraeni	SITH ITB
17.	Dr. Rizkita Rahmi Esyanti	SITH ITB
18.	Dr. Anggraeni Barlian	SITH ITB
19.	Dr. Ramadhani Ekaputra	SITH ITB
20.	Dr. Rina Ratnasih	SITH ITB
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22.	Ihak Sumardi, PhD	SITH ITB
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24.	Dr. Trimurti Wardhini	SITH ITB
25.	Dr. Fenny M. Dwivany	SITH ITB
26.	Dr. Rijanti Rahayu Maulani	SITH ITB
27.	Dr. Ayda T. Yusuf	SITH ITB
28.	Dr. Sri Harjati Suhardi	SITH ITB
29.	Dr. I. Nyoman P. Aryantha	SITH ITB