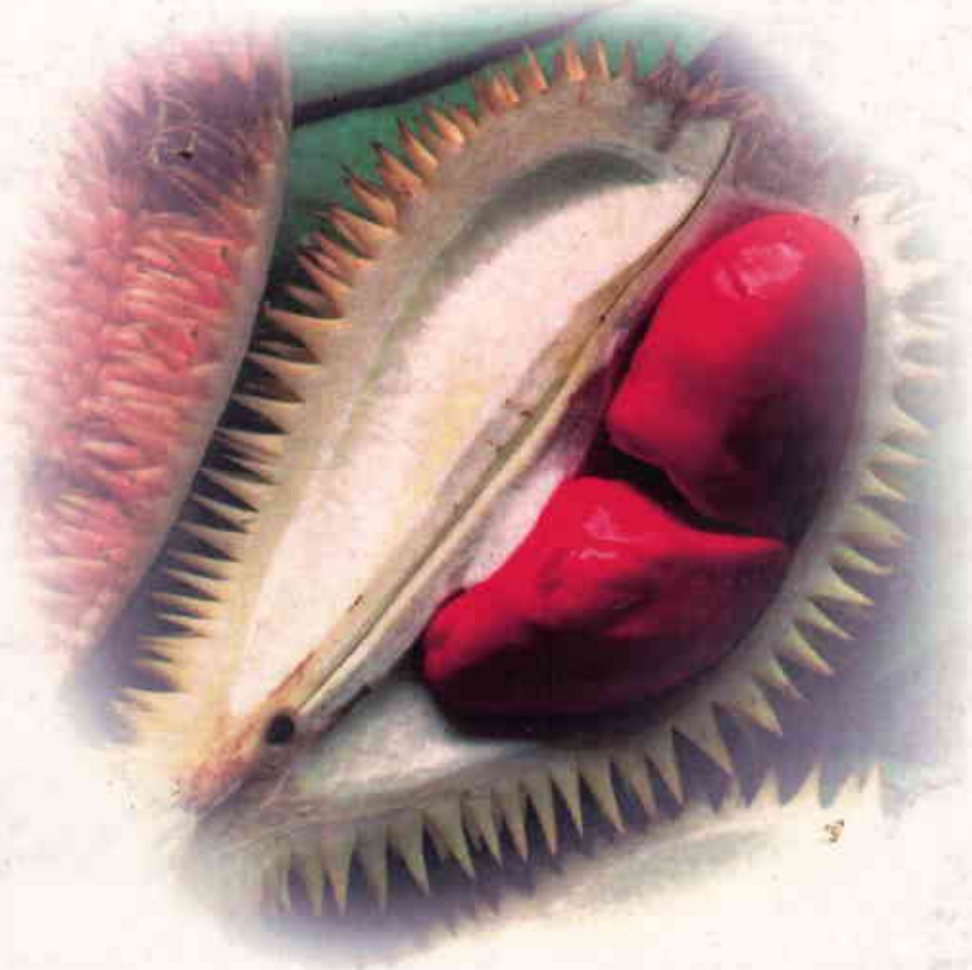
PROCEEDINGS OF NP-SEA



NATURAL PIGMENTS CONFERENCE FOR SOUTH-EAST ASIA (NP-SEA)

MALANG

MARCH, 20TH - 21ST 2010

ORGANIZED BY

MA CHING RESEARCH CENTER FOR PHOTOSYNTHETIC PIGMENTS (MRCPP),
MA CHUNG UNIVERSITY, MALANG, EAST JAVA, INDONESIA

IN COOPERATION WITH

THE BUREAU OF PLANNING AND INTERNATIONAL COOPERATION,
MINISTRY OF NATIONAL EDUCATION
JAKARTA, INDONESIA

MASTER OF BIOLOGY PROGRAM, SATYA WACANA CHRISTIAN UNIVERSITY

PROCEEDINGS OF
NATURAL PIGMENTS CONFERENCE FOR SOUTH-EAST ASIA (NP-SEA)

MARCH 20TH – 21ST, 2010
BALAI PERTIWI BUILDING
MA CHUNG UNIVERSITY, MALANG, INDONESIA

PROCEEDINGS

NATURAL PIGMENTS CONFERENCE FOR SOUTH-EAST ASIA (NP-SEA) MALANG, MARCH, 20TH – 21ST 2010

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Organized by

Ma Chung Research Center for Photosynthetic Pigments (MRCPP), Ma Chung University, Malang, East-Java, Indonesia

In cooperation with

The Bureau of Planning and International Cooperation, Ministry of National Education Jakarta, Indonesia

Master of Science in Biology Program, Satya Wacana Christian University

Welcome to Natural Pigments Conference for South-East Asia 2010

Since 2007 the Indonesian pigment researcher community has held two national scientific seminars on *Back to Nature with Natural Pigments* (Salatiga, 2007) and *Science and Technology of Natural Pigments* (Salatiga, 2008) fully supported by BPKLN of the Ministry of National Education, the Republic of Indonesia. From year to year, people who show interest and researchers in biopigment have increased tremendously, as evidenced by the involvement of researchers from different educational institutions as well as research institutions in Indonesia. At the Second National Seminar on Pigment at Hotel Quality, Salatiga, Dr. Ferry F. Kawur and Dr. Leenawaty Limantara proposed the idea of the establishment of *Himpunan Peneliti Pigmen Indonesia* (HP2I) (The Association of Indonesian Pigment Researchers) which the majority of the seminar participants supported. It is expected that the association would strengthen the existence of the natural pigment researchers and practitioners and the contribution of their ideas, work, and real contribution to Indonesian society. In addition, it is also anticipated that the association will become a scientific power in biopigment in South-East Asia.

In the course of time, Ma Chung Research Center for Photosynthetic Pigments, Ma Chung University, collaborated with the Master's Program in Biology, Universitas Kristen Satya Wacana, Salatiga and BPKLN, Ministry of National Education, the Republic of Indonesia, decided to hold an NP-SEA 2010 for South-East Asia. It is expected that through this seminar, the pigment researchers in South-East Asia can exchange ideas, build a network, and strengthen the existence of biopigment research in South-East Asia. The keynote speaker, Prof. Hugo Scheer, from Ludwig-Maximilian University, Munich, Germany, as an authority on photosynthetic pigment, together with Prof. Leszek Fiedor, from Jagiellonian University, Poland, were invited to widen the horizon of Indonesian researchers on the rapid development of pigment research in the world, and particularly in Europe. Positive responses on the seminar came from researchers in India, the Philippines, Nigeria, Serbia, and Russia, but the biggest obstacle is the travel expenses and expectations of the researchers to get financial support from the committee. There is as yet response from the closest countries such as Singapore, Malaysia, New Zealand, and Timor Leste. The committee earnestly hope that in the Second NP-SEA, a number of problems that arise can be overcome by the involvement of world organizations as supporters of this seminar.

To conclude, with all our shortcomings, the committee wish you all the best in the seminar. May this opportunity become the theatre to strengthen networking, widen horizon, and be aware of the development of biopigment research in Indonesia in particular, and in the world in general.

Malang, 20 March 2010,

Leenawaty Limantara
Chairperson

Greetings from the Coordinator of the Bureau of Planning and International Cooperation – Ministry of National Education, Indonesia

On behalf of the Bureau of Planning and International Cooperation, the Ministry of National Education, Indonesia we are honoured to welcome you to the Natural Pigments Conference for South-East Asia (NP-SEA) 2010 held in March, 20-21st, 2010 at Ma Chung University, Malang, Indonesia. The implementation of the *Beasiswa Unggulan* Program (Excellent Scholarship Program) from the Ministry of National Education, Indonesia, for graduate students majoring in chlorophylls and natural pigments at the Magister of Biology Program, Satya Wacana Christian University, started the ball rolling, and we have observed and obtained a great development of the Scholarship Program that four batches of graduate students have enjoyed.

The success of earlier national seminars on Pigments in 2007 and 2008 has led to the International Seminar called *NP-SEA 2010* which is followed by a breakthrough in increasig the level of speakers, from national speakers to international ones. This has become a great achievement which should be further developed in the upcoming years.

On behalf of the Bureau of Planning and International Cooperation, the Ministry of National Education, Indonesia, I would like to congratulate the committee as well as the participants of NP-SEA 2010 on your success in organizing and participating in the NP-SEA 2010. I hope this international seminar could be an opportunity to share information, to build a cooperation network, and to strengthen the existence of The Association of Indonesian Pigment Researchers, HP2I. I do appreciate the conference and hope also that the NP-SEA will be conducted again in 2012 in Indonesia or one of South-East Asian countries. Thus it will be held regularly as an annual or biannual meeting. Finally I wish you "*Selamat Berkonferensi*" and "*Selamat Berkarya*" (enjoy your conference and be fruitful in your scientific life)

Jakarta, March 1st, 2010

DR. R. Agus Sartono, MBA
Head
The Bureau of Planning and International Cooperation
Ministry of National Education
Indonesia

Schedule of NP-SEA 2010

Time	Program
Day 1 : 20th March, 2010	
07:45 – 08:30	Registration
08:30 – 09:00	Opening Ceremony and Welcome Dance
09:00 – 09:15	Short Speech by BPKLN Representative
09:15 – 10:30	Topic: *Chlorophylls: from Photosynthesis to Photodynamic Therapy* by Prof. Hugo Scheer (Germany) Topic: Interaction of Chlorophylls with Animal Organisms by Prof. Leszek Fiedor (Poland)
11:15 – 11:30	Coffee Break
11:30 – 12:15	Plenary Speakers: 1. Dr. Ocky Karna Radjasa 2. Ir. Ferry F. Karwur, Ph.D.
12:15 – 12:45	Inauguration of HP2I (*Himpunan Peneliti Pigmen Indonesia*) – *The Association of Indonesian Pigment Researchers*
12:45 – 13:00	Photo Session
13:00 – 14:00	Lunch Break
14:00 – 16:00	Poster Presentation and Scientific Expo
16:00 – 16:30	Coffee Break
16:30 – 17:00	Closing Ceremony and Announcement of the Winners of Poster Presentation
17:00 – 18:00	Dinner
Day 2: 21st March, 2010	
00:00	Depart to Mount Bromo
03:00 – 03:30	Estimated arrival at Penanjakan Sunrise View Area
06:00 – 06:30	Heading for Bromo Crater
09:00 – 10:00	Breakfast
10:00 – 12:00	Return to Malang

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B.07

Influence of the Length of Evaporation Time of *Angkak* Extract on the Redness and Antimicrobial Activity against *Bacillus subtilis*

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Abstract

Red, orange and yellow pigments are produced by *Monascus* fungi during fermentation in *angkak* production. Many studies showed that it also produced other metabolites which have antimicrobial activity. The influence of the length of evaporation time of *angkak* extract on the redness and antimicrobial activity against *Bacillus subtilis* has been studied. *Angkak* was extracted using water at proportion of *angkak* and water of 1:4 w/v. The obtained extract was evaporated using vacuum rotary evaporator at 50°C, 50 mbar at different lengths of evaporation time i.e. 15, 30, 45, 60 and 75 minutes. The redness and antimicrobial activity against *Bacillus subtilis* of evaporated extract were determined. The redness was measured by lovibond tintometer, whereas antimicrobial activity against *Bacillus subtilis* was determined using contact method. The data were analyzed statistically using ANOVA at α=5% and followed by Duncan's Multiple Range Test (DMRT) at α=5%. The redness increased as the time of evaporation was extended. Evaporation time of 75 minutes resulted in the highest redness i.e. 10.58. The longer time of evaporation up to 60 minutes resulted in the increase of antimicrobial activity. However, when longer time was extended further, it decreased its antimicrobial activity. Evaporation time of 45 minutes reduced the total of *Bacillus subtilis* from 8.168 to 7.059 log cfu.

Keywords: *angkak* extract, evaporation, redness, antimicrobial activity, *Bacillus subtilis*

Introduction

Angkak or *red yeast rice* is a product of rice fermentation by *Monascus sp* fungi. During fermentation, *Monascus sp* produced red, orange and yellow pigments, therefore *angkak* has been used for colouring food and beverage. Other than pigments, *Monascus sp* can also produce some other metabolites, e.g. Monacolin and its homolog, Monascidin A and some enzymes such as α-amylase, β-amylase, glucoamylase, α-galactocydase, polypectase, protease and lipase. *Monascidin* is pentaketyde which has antimicrobial activity against Gram positive bacteria (Wong & Bau, 1977).

In the market, *angkak* is commonly found in the form of whole rice or *angkak* flour. *Angkak* rice and flour are simple to produce, but limited in the application as colouring agent in food because of its high starch content. Extraction of the monascus pigment will expand the application to food. Evaporation of *angkak* extract provides some advantages: more practical in the application, and it simplifies the packaging, storage and distribution. Longer evaporation will produce higher concentration of substances of *angkak* extract, therefore colour intensity and antimicrobial activity will also increase. However, overly long evaporation could reduce the colour intensity and antimicrobial activity (Jenie et al, 1997). Vacuum evaporation can lower the evaporation temperature, so it can reduce the detrimental effects of higher temperature.

Preliminary research showed that evaporation of *angkak* extract to half volume using rotary vacuum evaporator resulted in evaporated *angkak* extract with antimicrobial activity against *Bacillus subtilis* of 2 log cycle reduction. *Bacillus subtilis* is decomposting bacteria commonly found in food. Evaporation time of the experiment was 50 minutes, therefore it could be estimated that the rate of evaporation was 2 mL per minute. The aim of this research was to study the influence of the length of evaporation time of *angkak* extract on the redness and antimicrobial activity against *Bacillus subtilis*.

Materials and Methods

Materials

Angkak (*red yeast rice*) was purchased from the Chinese traditional medicine shop *Ban Zhe Tong*, Jalan Jagalan, Surabaya, East Java, Indonesia. *Angkak* was packed in a sealed plastic and stored in refrigerator (8-10

°C). Pure culture of *Bacillus subtilis* was obtained from the Microbiology Laboratory, Widya Mandala Surabaya Catholic University. Nutrient agar (Merck 1.05450), nutrient broth (Merck 1.05443), peptone broth 0,1% (Merck 1.07224) aquadest and chemicals were procured from a local distributor in Surabaya.

Methods

Flow chart of the research implementation is shown in Figure 1. Extraction was done by mixing *angkak* with water (1:4 w/v) in an Erlenmeyer, then shaken at shaking waterbath (GFL 1083) at 40°C for 30 minutes. *Angkak* extract of 200 mL was evaporated using *rotary vacuum evaporator* at 50 °C and 50 mbar. The length of evaporation time varied i.e. 15; 30; 45; 60 and 75 minutes. Randomized block design experimental research was used in this research. The experiment was done in four replications.

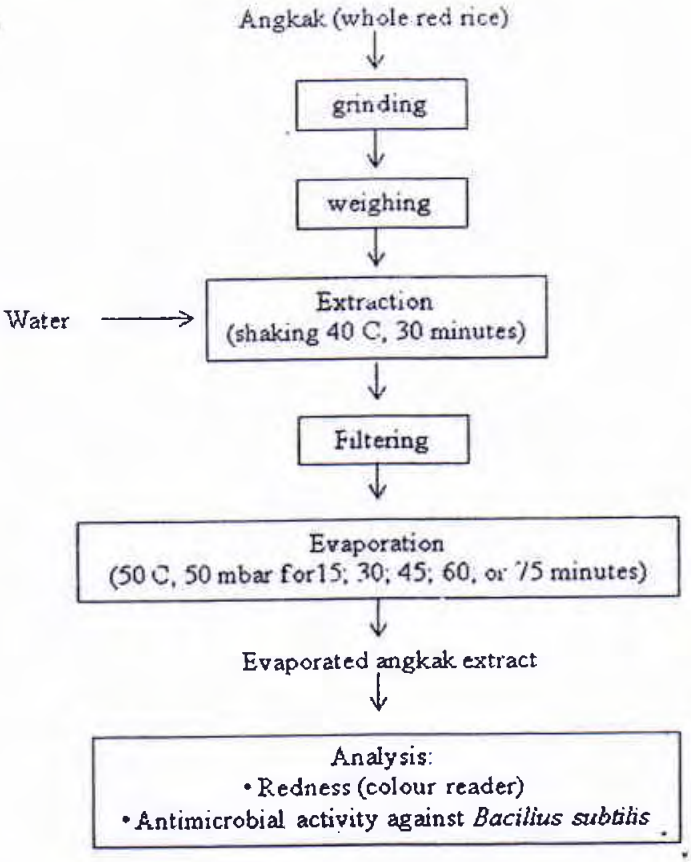

Figure 1 Flow chart of the research implementation

Redness measurement

Redness (red intensity) was measured by *color-reader* (Minolta). The colour measurement of evaporated *angkak* extract resulted in 3 parameters i.e. L, a and b, where L is Lightness, a is redness and b is yellowness. This experiment only used redness data.

Analysis of antimicrobial activity against Bacillus subtilis

Analysis of antimicrobial activity against *Bacillus subtilis* using contact method. 0,5 mL of broth culture of *Bacillus subtilis* which equals to 1,5 x 10^8 cfu (colony forming unit) was contacted with *angkak* extract of 4,5 mL for 30 minutes. The schematic procedure was shown in **Figure 2**.

Data Analysis

The obtained data were statistically analyzed using *Analysis of Varians*, followed by *Duncan's Multiple Range Test* at $\alpha = 5\%$

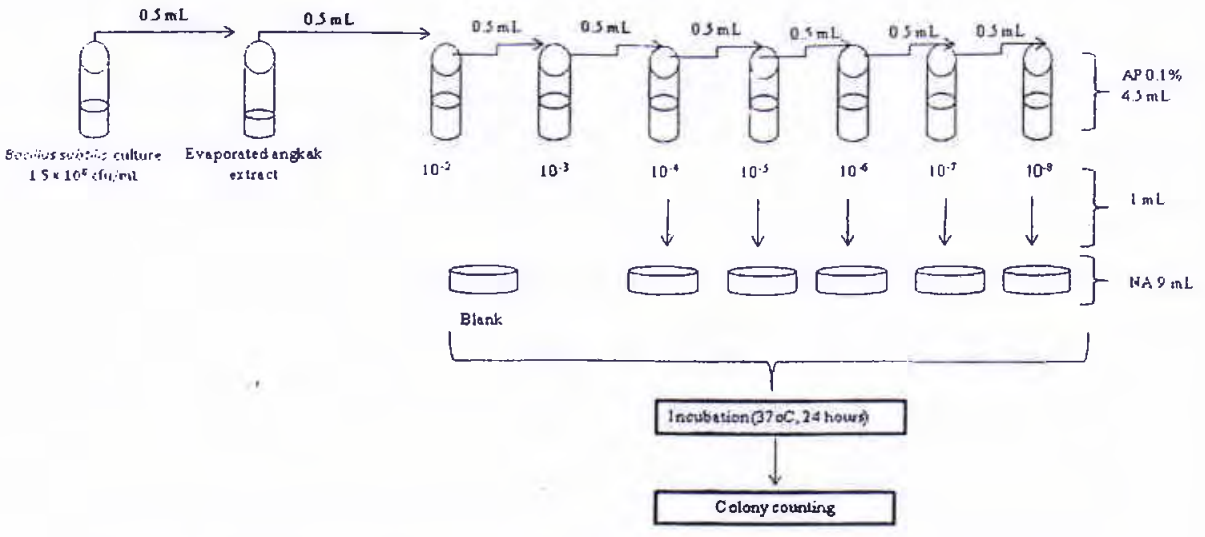

Figure 2 Schematic procedure of analysis of antimicrobial activity of evaporated *angkak* extract

Results and Discussion

Influence of the length of evaporation time of *angkak* extract on the redness

Figure 3 showed the influence of the length of evaporation time of *angkak* extract on the redness. The redness increased as the time of evaporation was extended. Evaporation time of 75 minutes resulted in the highest redness, i.e. 10.58. During evaporation, water of *angkak* extract vaporized, therefore pigment concentration increased. Increasing pigment concentration resulted in the higher redness of evaporated *angkak* extract. The redness reflected the level of *monascorubrin* and *rubropunctatin*, the dominant red pigments of *angkak* extract. Redness of evaporated *angkak* extract was in the range of 6.73 and 10.58. The redness of *angkak* extract of evaporation time of 15 until 60 minutes was not different statistically. Evaporation time of 75 minutes resulted in the higher significance of the redness than that of 60 minutes. Higher redness reflected the higher potentiality of *angkak* extract as food colorant. Higher redness will reduce the amount of *angkak* extract to be applied as colorant for food.

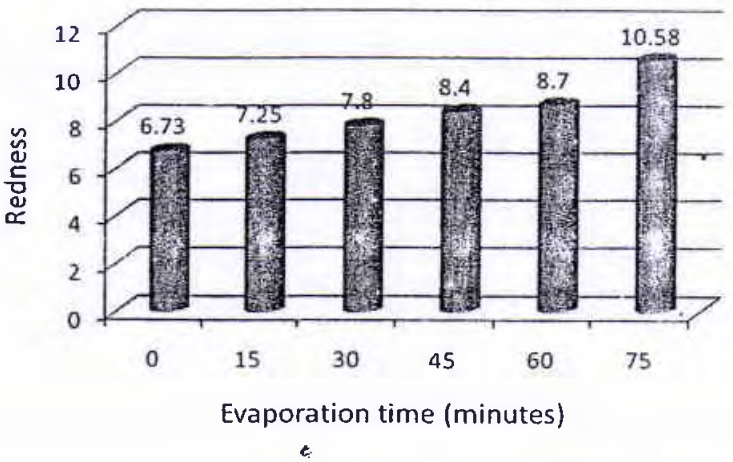

Figure 3 Influence of the length of evaporation time of *angkak* extract on the redness

Mean value of total count of *Bacillus subtilis* after coming into contact with the *angkak* extract at different evaporation times were shown in figure 4.

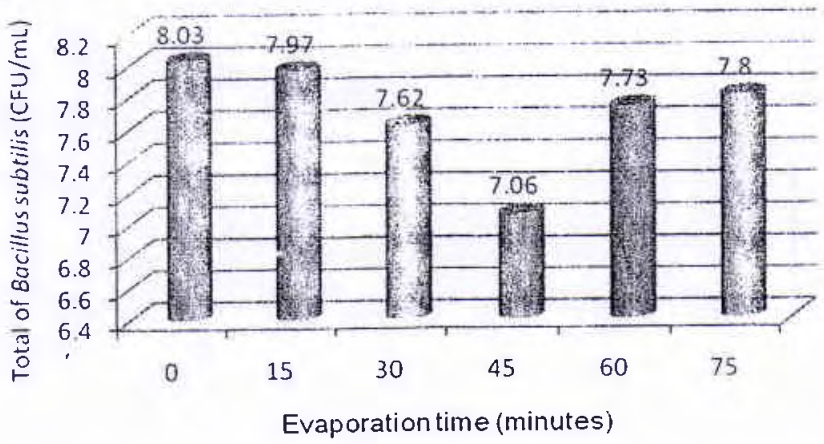

Figure 4 Influence of the length of evaporation time of *angkak* extract on the antimicrobial activity against *Bacillus subtilis*

Based on ANOVA at α = 5% and DMRT at α = 5%, different evaporation times gave a significant effect on the antimicrobial activity against *Bacillus subtilis*. Total count of *Bacillus subtilis* of *angkak* extract without evaporation was 8.030 log cfu/ mL which was not significantly different from the extract *angkak* with 15 minutes. However, the total count of *Bacillus subtilis* was significantly different from the evaporation time of 30; 45 and 60 minutes. Evaporatin time of 45 minutes resulted in the lowest of total count of *Bacillus subtilis*. The longer time of evaporation up to 60 minutes resulted in the increase of antimicrobial activity. However, much longer time decreased its antimicrobial activity. Evaporation time of 45 minutes reduced the total of *Bacillus subtilis* from 8.168 to 7.059 log cfu.

Conclusions

The redness increased as the time of evaporation is extended. Evaporation time of 75 minutes resulted in the highest redness, i.e. 10.58. The longer time of evaporation up to 60 minutes resulted in the increase of antimicrobial activity. However, much longer time decreased its antimicrobial activity. Evaporation time of 45 minutes reduced the total of *Bacillus subtilis* from 8.168 to 7.059 log cfu.

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