3rd NPSEA

Conference Program & Extended Abstract

The Natural Pigments Conference for South-East Asia
In Conjunction with: LCMS WORKSHOP from SHIMADZU

RESEARCH AND DEVELOPMENT OF PIGMENT-BASED INNOVATION AND TECHNOLOGY

22-23 AUGUST 2016

The Core R&D Center, Universitas Ma Chung

Malang, East Java, Indonesia

Organized by:

Universitas Ma Chung

SHIMADZU Excellence in Science

Partners:

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Ikatan Apoteker Indonesia IAI

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DAAD Deutscher Akademischer Austausch Dienst

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PREFACE

The 3rd Natural Pigment Conference for South-East Asia (NP-SEA) Secretariat Office
Ma Chung Research Center for Photosynthetic Pigments (MRCPP)
Universitas Ma Chung, Villa Puncak Tidar N01, Malang 65151 East Java, INDONESIA
Tel./Fax +62 341 550 171/175, E-mail: mrcpp@machung.ac.id

Opening Remark from The Chairman of The 3th-NPSEA

Dear researchers and friends,

On behalf of the organizing committee, I would like to give you a warm welcome in the 3rd Natural Pigments Conference for South-East Asia (NP-SEA) 2016.

Natural pigments are the most obvious and eye-catching substances that can be found in flowers, leaves, bird feather, algae, photosynthetic bacteria, and many more. These pigments have been used as bountiful colorants for food, cosmetics, and textiles and very close connected to the culture of South-East Asia. If we look closely, pigments such as chlorophylls and carotenoids play importance role as key pigments, which capture radiant of energy from the Sun in the process called photosynthesis – a process that convert solar energy into fuels. In agriculture, the natural pigments are important photosensors and indicators for health status. There are many applications that have been revealed through the study of structure and function of natural pigments.

We are looking forward of your active participation during the Natural Pigment Conference for South-East Asia to present your works, to raise questions, and triggers discussion on the recent research and development of pigment-based innovation and technology. We are inviting high profile scientists and practitioner in the industry as the keynote and plenary speakers. We wish that their presence would be a great encouragement and motivation for students and young researchers in the South-East Asia to take part in the research and development of natural pigments.

We are very happy to have 125 participants including the keynote speaker, plenary speakers, invited speakers, poster speakers, students and other participants from Germany, Switzerland, France, US, the Philippines, Singapore, and Indonesia. In this event, we would like to extend our acknowledgement to our partners, who support us financially, i.e. Kemenristekdikti, DAAD, ITS Scientific as well as PT Ditek Jaya and Shimadzu (Asia Pacific) Pte Ltd. We thanks to the Indonesian Society Pigment Researchers (Himpunan Peneliti Pigmen Indonesia, HP2I) for cooperation as steering committee, the Indonesia Pharmacist Association (Ikatan Apoteker Indonesia, IAI) for certifying this event with 6 credit points, the Indonesian Chemical Society (Himpunan Kimia Indonesia, HKI) and Indonesian-German Network (IGN) for disseminating this event to their members. We thanks also to Universitas Ma Chung for having this venue with superb facilities and supports from the staffs.

I do hope that you will find your time here enjoyable and a source of many insights that will help to advance the understanding of natural pigments and to encourage the collaborations and friendship, scientific exchange, the development of joint interests and project that are of scientific and economic importance in order to exploit the natural pigments and their importance in the most aspect of living, e.g. food and health, fashion, agriculture and advanced technologies.

Thank you very much and please enjoy this event.

Yours sincerely,

Tatas H.P. Brotosudarmo, Dipl.Chem., Ph.D

Chair of the NP-SEA 2016

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Opening Remark from The Rector of Universitas Ma Chung

Dear Friends, participants of the Natural Pigments Conference for South East Asia 2016.

Welcome to Universitas Ma Chung, welcome to Malang, a beautiful city in Indonesia. We are happy that natural pigments experts from several countries, Indonesia, German, Switzerland, USA, France, the Philippines and Singapore gathered together here to share their knowledge and research results. We hold the NP Conference South East Asia this year in Universitas Ma Chung, Malang. It is not by chance that the international conference is holding here, because Universitas Ma Chung has an expertise in Natural Pigments which is institutionally embodied as Ma Chung Research Center for Photosynthetic Pigments (MRCPP). MRCPP is not only supported by Universitas Ma Chung but also by Indonesian government by recognizing MRCPP as one of the national scientific center of excellence.

Indonesia is one of the countries with a rich biodiversity, and consequently rich of natural pigments source. However, natural pigments industry in Indonesia and South East Asia is still lag behind the developed countries, whereas it is expected that the demands of natural pigments in various industries will increasing in the future. Therefore the research on natural pigments is one of the important research field. Indonesian government is also promoting the dissemination of research results to be applied in industry, not only finish in scientific publication.

Many kind of natural pigments in Indonesia and their properties are still unknown, they are remains to be investigated to provide benefit for human welfare. The international research cooperation in natural pigments will accelerate the rate of discovery and innovation in applying the knowledge for human welfare. Therefore, this conference is an important conference not only for the pigments research society but also for other research field and industry.

Besides the conference I hope the participants can also enjoy the natural beauty of Malang and its historic heritage.

I hope you enjoy staying in Malang, obtain a great benefit from the conference, and develop cooperation framework with other conference participants.

I wish to thank the speakers, poster presenters, students, and other attenders for attending the conference also partners who supported the conference.

Malang, August 18, 2016

Rector of Universitas Ma Chung

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GENERAL SCHEDULE
THE 3rd NATURAL PIGMENTS CONFERENCE FOR SOUTH-EAST ASIA (NP-SEA) 2016
August 22, R&D Center Universitas Ma Chung

Monday, August 22, 2014		
The 3rd Natural Pigments Conference for South-East Asia (NP-SEA) 2016		
Time	**Program**	**Room**
07:00 – 08:00	Registration	R&D Center 1st floor
08:00 – 08:10	Opening Remark by Conference Chairman by Tatas H. P. Brotosudarmo, Ph.D.	R&D Center Hall 6th floor
08:10 – 08:45	Keynote Speaker : ***[Chlorophylls: From Photosynthesis to Photodynamic Therapy]*** by Prof. Dr. Hugo Scheer (Moderator : Leenawaty Limantara, Ph.D.)	R&D Center Hall 6th floor
08:45 – 09:10	Plenary Speaker : ***[Chlorophyll Breakdown During Leaf Senescence: A Novel Role for TIC55 as a Hydroxylase of Phyllobilins, the Products of Chlorophyll Breakdown]*** by Prof. Stefan Hörtensteiner (Moderator : Leenawaty Limantara, Ph.D.)	R&D Center Hall 6th floor
09:10 – 09:35	Plenary Speaker : ***[The Untapped Richness of Pigment-producing Marine Organisms and Their Associants]*** by Prof. Ocky Karna Radjasa (Moderator : Leenawaty Limantara, Ph.D.)	R&D Center Hall 6th floor
09:35 – 10:00	Plenary Speaker : ***[Marine Fungal Pigments Diversity and Potential Use]*** by Dr. Kustiariyah Tarman (Moderator : Leenawaty Limantara, Ph.D.)	R&D Center Hall 6th floor
10:00 – 10:15	Coffee break	R&D Center Hall 4th floor
10:15 – 10:50	Keynote Speaker : ***[Potential Market of Pigments in Daily Life: Food, Health and Fashion in Indonesia]*** by Ir. Thomas Darmawan (Moderator : Ferry F. Karwur, Ph.D.)	R&D Center Hall 6th floor
10:50 – 11:15	Plenary Speaker : ***[Review on the Metabolites of Monascus]*** by Dr. Philippe J. Blanc (Moderator : Ferry F. Karwur, Ph.D.)	R&D Center Hall 6th floor
11:15 – 11:40	Plenary Speaker : ***[Nexera UC, New Concept On-pine SFE-SFC-MS: Principles and Applications of SFE-SFC-MS/MS]*** by Dr. Xing Jie (Moderator : Ferry F. Karwur, Ph.D.)	R&D Center Hall 6th floor
11:40 – 12:05	Plenary Speaker : by Prof. Sherry A. Tanumihardjo, Ph.D. (Moderator : Ferry F. Karwur, Ph.D.)	R&D Center Hall 6th floor
12:05 – 13:30	Lunch	R&D Center Hall 4th floor

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Time	Program	Room
13:30 – 14:45	Invited speaker – Session 1	R&D Center 6th floor (Class A)
		R&D Center 6th floor (Class B)
14:45 – 15:45	Coffee Break Poster Session	R&D Center Hall 4th floor
15:45 – 16:45	Invited speaker – Session 2	R&D Center 6th floor (Class A)
		R&D Center 6th floor (Class B)
16:45 – 17:15	3rd NP-SEA Awards for Best Poster and Closing Remark	R&D Center Hall 6th floor
17:15 – 18:00	Preparation for Gala dinner	-
18:00 – 20:00	Gala dinner	Balai Pertiwi

Tuesday, August 23, 2014

Shimadzu Advance Liquid Chromatography Mass Spectrometry (LCMS/MS) Technologies and Applications Workshop in Junction with The Natural Pigments Conference for South-East Asia

Time	Program	Room
07:00 – 8:00	Registration	MRCPP R&D Center Hall 3rd floor
08:00 – 09:00	Session 1 : Introduction of LCMS/MS and Application	
09:00 – 12:00	Session 2 : Technical Workshop (I)	
12:00 – 13:00	Lunch	
13:00 – 16:00	Session 3 : Technical Workshop (II)	
16:00 – 16:15	Clossing	

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Schedule of Oral Presentation

Oral Presentation – Session 1			
Meeting Hall at 6th Floor of The R&D Center (Class A) **Moderator: Dr. Ir. Edia Rahayuningsih, M.S.**			
Time	**Authors**	**Title**	**Code**
13:30-13:45	Delianis Pringgenies, Riyanda Idris, Muhammad Zainudin	The Antioxidant Activity of Carotenoid Pigments in the Bacterial Symbionts of Seagrass *Syringodium isoetifolium*	ON-01
13:45-14:00	Victor Aprilyanto, Andrea Putri Subroto, Chris Darmawan, Reno Tryono, Condro Utomo, and Tony Liwang	In Vitro Selection of single guide RNA for Effective Cleavage of Exon-3 VIRESCENS Gene in Oil Palm Using CRISPR/Cas9 System	ON-02
14:00-14:15	Abdullah Muzi Marpaung, Nuri Andarwulan, Purwiyatno Hariyadi and Didah Nur Faridah	The Color Stability of Butterfly Pea (*Clitoria ternatea* L.) Petal Extract at pH 6 to 8 are Highly Uncertain	ON-03
14:15-14:30	Mohammad Junus	Algae Cells Density in Various Planting Period and Liquid Sludge Biogas Unit Proportion	ON-04
14:30-14:45	Uun Yanuhar	The Involvement Fragment Pigment Protein (FPP) Microalga *Nanochloropsis oculata* of Response Heat Shock Protein 70 (HSP70) of Infection Nervous Necrotic Viral (NNV) on Grouper	ON-05
Room at 6th floor of The R&D Center (Class B) **Moderator: Prof. Erlinda A. Vasquez**			
13:30-13:45	Windu Merdekawati	The Uniqueness of Seaweed Pigments	ON-06
13:45-14:00	Ermiziar, T., Saragih, R., Hanum, L.	Natural Pigment from Red Colour Melinjo Peels	ON-07
14:00-14:15	Pujiyanto, Muhammad Iqbal Prawira Atmadja and Dadan Rohdiana	Theaflavin, Natural Pigment on Black Tea and Its Pharmacological Activities	ON-08
14:15-14:30	Failisnur, Sofyan and Anwar Kasim	Dyeing of Cotton Fabric with Natural Dye from Gambier (*Uncaria gambir* Roxb.)	ON-09
14:30-14:45	Defri Yona and Park Mi Ok	Seasonal variation of phycoerythrin chromophores of *Synechococcus* spp. in the East Sea, Korea	ON-10

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Oral Presentation – Session 2			
Meeting Hall at 6th floor of The R&D Center (Class A) Moderator: Mr. Victor Aprilyanto			
15:45-16:00	Darda Efendi, H. Muthmainnah, T.S. Arzam, I. H. Sumiasih, R. Poerwanto, and Y.A. Purwanto, A. Agusta, and S. Yuliarni	Degradation of Chlorophyll and formation of β-cryptoxanthin and ß-citraurin in Citrus Degreening	ON-11
16:00-16:15	Edia Rahayuningsih	The Sustainable Economic Development through Research, Production, and Application of Natural Dye	ON-12
16:15-16:30	Delicia Yunita Rahman, Dwi Susilaningsih and Marc J.E.C. van der Maarel	Heterotrophic growth of LIPI13-AD014 for Phycocyanin Production	ON-13
16:30-16:45	Muh. Thoyib, Catur Harsito, Suyitno, Syamsul Hadi	Simple Procedure for Reducing Cratering Defect of Water-Based Paint Using *Caesalpinia Sappan* Dye	ON-14
Room at 6th floor of The R&D Center (Class B) Moderator: Dr. Dadan Rohdiana			
15:45-16:00	Anna Yuliana, Marlia Singgih Wibowo, Elin Julianti	Toxicity Level of Monascus Pigments Using Ecosar Program	ON-15
16:00-16:15	Erlinda A. Vasquez,Candelario L.Calibo, Ronnel M. Godoy and Lady Fatima G. Palermo	Alteration of the Chlorophyll Content in Phytoplasma-Infected Cassava	ON-16
16:15-16:30	Rika Wahyuningtyas & Uun Yanuhar	The Expression of MHC Class 1 in *Cyprinus carpio* Infected Koi Herpes Virus through Induction of Crude Protein from Macroalgae *Halimeda* sp	ON-17
16:30-16:45	Mada Triandala Sibero, Kustiariyah Tarman, Rita Sahara	Exploration of Red Pigment from Coastal Endophyte Fungi Isolated from *Hydnophytum formicarum*	ON-18

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Poster Presentation Room at 4th floor of The R&D Center 14:45 – 15:45			
No	**Authors**	**Title**	**Code**
1	Elfi Anis Saati, Sita Ayu Pangesti, Sri Winarsih and Moch. Wachid	Co-pigmentation Anthocyanins of Rose Pigment (varieties of Batu Local) with Catechin from Black Tea and Green Tea Extracts	PN-01
2	Andreas Lucky Effendy, Rollando	In silico screening study of potent human breast cancer drug from natural pigments	PN-02
3	Antonius Herry Cahyana, Kam Natania and Hong Fu Sheng	Study on Antioxidant Activity, Binding Capacity and Stability of Curcumin-Functionalized Fe_3O_4 Magnetic Nanoparticles	PN-03
4	Ayda Krisnawati and M. Muchlish Adie	Consistency of Biomass Production from Several Soybean Genotypes in Various Agro Ecology of Indonesia	PN-04
5	Diah Mustika Lukitasari, Rosita Dwi Chandra, Heriyanto, Renny Indrawati	Stability and Antioxidant Activity of Microencapsulated Pigment from Red Spinach (*Amaranthus tricolor*) for Food Colourants	PN-05
6	Elin Julianti, Laida Neti Mulyani,Marlia Singgih Wibowo, Susanti	Comparison Different Extraction method of C-Phycocianin, a Phycobiliprotein from Dry Biomass of *Spirullina platensis*	PN-06
7	Ervika Rahayu NH, Dini Ariani, Miftakhussolikhah, Maharani P.E., Yudi P	The Effect of Yellow Natural Color from Turmeric on Physical and Sensory Properties of Arenga Starch-*Colocasia Esculanta* L. Noodle	PN-07
8	Giacinta Mutiara Beta Maharani, Filiana Santoso, and Abdullah Muzi Marpaung	Stability Improvement of Anthocyanin from Various Local Plants using Metal Complexation	PN-08
9	Kam Natania, Antonius Herry Cahyana, Melanie Cornelia dan Edison Sutiyono	Microencapsulation of Soursop (*Annona muricata* Linn.) Leaf Tea Extract Using Natural Mucilages	PN-09

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Poster Presentation Room at 4th floor of The R&D Center 14:45 – 15:45			
No	**Authors**	**Title**	**Code**
10	M. Muchlish Adie and Ayda Krisnawati	Identification and Clustering Soybean Genotypes with High Biomass Production as a Source of Renewable Energy	PN-10
11	Melanie Cornelia and Oktafielia Putri	Application of Goji Berry Fruit (*Lycium barbarum* L.) extract as Food colorant in Dried Noodle	PN-11
12	Miftakhussolikhah, Dini Ariani, Ervika RNH, Azkia Nastiti, Yudi Pranoto	Effect of Additional Suji Leaves and Turmeric Extract on Physicochemical Characteristic and Antioxidant Activity of Arenga-Canna Noodle	PN-12
13	Selfina Gala, Dhaniar Rulandri Widoretno, Delita Kunhermanti, Lailatul Qadariyah, Sumarno and Mahfud	Microwave-assisted Extraction of Natural Dyes from Jackfruit Wood Waste (*Artocarpus heterophyllus* Lamk)	PN-13
14	Renny Indrawati, Gita, Kristine, Melissa, Yuyun Yuniati, Leenawaty Limantara	How extensive does the artificial dye color our food?	PN-14
15	Swanty Rahmazania Mustika and Abdullah Muzi Marpaung M.P	Color properties and Stabilizing Effect of Metal ion on Blue Anthocyanin Color from Buni (*Antidesma bunius*) Fruit	PN-15
16	Rosita Dwi Chandra, Renny Indrawati, Mario Sent Anugrah, Jodiawan, Ricky Santoso, Tatas H. P. Brotosudarmo, Leenawaty Limantara	Uncovering the Availability of Products Enriched with Vitamin A in Local Supermarket	PN-16

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Poster Presentation Room at 4th floor of The R&D Center 14:45 – 15:45			
No	**Authors**	**Title**	**Code**
17	Yudi Purnomo, Fajar Audra Pratama, Nur Rohman	Hepatoprotector and Anti-Hemolysis Activity of Tommato (*Lycopersicon pimpinellifolium*) Juices In Rats Induced Alum	PN-17
18	Endang Kusdiyantini, Iffan Alif, Salma Fuadiyah, Dyah Wulandari, Anto Budiharjo	Identification of Red-Pigmented Thermophile Bacteria Isolated from Gedong Songo Hot Spring, Semarang – Central Jawa	PN-18
19	Setiyono, E., Pringgenies., Heriyanto, Prihastyanti, M.N.U, Shioi, Y., Brotosudarmo, T.H.P	Carotenoid Analysis from *Erythrobacter flavus* Symbiont of *Acropora nasuta*	PN-19
20	Husnatain, I.D., Salim, K.P., Heriyanto, Purwantiningrum, I., Harijono, Limantara, L.	Effect of Dried Fruit Processing on Lycopene Content and Pigment Composition of Tommato (*Lycopensicum esculentum* var Marta)	PN-20
21	Wibowo, A.A., Elim, P.E., Heriyanto, Prihastyanti, M.N.U, Shioi, Y., Brotosudarmo, T.H.P	Effect of Drying Treatments on the Concentration of Fucoxanthin and Chlorophyll *a* and Pigment composition of Three *Sargassum* Species	PN-21
22	Yuyun Yuniati, Renny Indrawati, Jovine, Tantiana, Wynona	Tracing the antioxidant-rich products in local groceries: naturalness, biofunctionality, and price	PN-22
23	Yuyun Yuniati, Juliana, Lidwina Angelica Soetantijo, and Ratna Yulianti Wijaya, Renny Indrawati	Preparation of Antioxidant Drinks from Mulberry *Morus nigra* L.	PN-23

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PN-20

Effect of Sweetened Dried Fruit Processing on Lycopene Content and Pigment Composition of Tomato (*Lycopersicum esculentum* var. Marta)

Ikrima Dzil Husnatain[a], Katarina Purnomo Salim[b], Heriyanto[b], Indria Purwantiningrum[a], Harijono[a]*, Tatas H. P. Brotosudarmo[b] and Leenawaty Limantara[b,c]**

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Abstract

Tomato is a fruit widely consumed and recognized from its nutritional substances, i.e. vitamin C and lycopene. Processed tomato products are primary source of dietary lycopene. In this study, potential of sweetened dried tomato as dietary lycopene source was investigated. The aim of this study is to determine effect of processing steps against composition of main carotenoids. The total lycopene content was determined by spectrophotometric method, while the identification and composition of main carotenoids were analyzed by HPLC. All *trans* isomer of lycopene was the dominant pigment in tomato. The most significant decrease of total lycopene and all *trans* isomer of lycopene occurred on the processing step 2 (steaming) to step 3 (soaking in honey). The decrease of content was also observed in other main carotenoids (phytoene, phytofluene, and β-carotene).

Keywords: dried fruit, HPLC, lycopene, pigment composition, tomato
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1. Introduction

Tomato is a well-growth type of fruit in Indonesia. Raw and processed tomato products such as tomato sauce, tomato paste, and tomato soup are good source of dietary lycopene. Lycopene is a member of carotenoids (lipid soluble pigment) that highly accepted by food industry due to its potential health effects as antioxidant [1]. There are few information about dried processed tomato product such as dehydrated tomato [2]. The purpose of this study was to explore sweetened dried fruit as dietary lycopene source.

2. Methodology

2.1. Preparation of Sweetened Dried Tomato

Tomatoes (*Lycopersicum esculentum* var. Marta) were sliced into the same size (eight triangle-shaped part of each tomato) and then the seeds were discarded. There were 4 processing steps for making sweetened dried tomato as follow (1) the tomato flesh was soaked in 2% $Ca(OH)_2$ solution for 2 hours, (2) the tomato flesh from step 1 was steamed at 60°C for 3 minutes, drained, and the skins were discarded, (3) the tomato flesh from step 2 was soaked in honey with the ratio 1 and 2 (w/v) for 5 hours at 20°C, (4) the tomato flesh from step 3 was dried in vacuum oven at 50°C and 27 Mbar for 15 hours as the final product.

2.2. Extraction of Crude Lycopene

The tomato flesh from each processing step was extracted with organic solvents (methanol, acetone, and THF) according to modified method of Fujii *et al.* [3]. 100% methanol and 100% acetone were used to remove water content in the sample, and followed by extraction with THF to extract crude carotenoids. Crude carotenoid was dried by N_2 gas and stored at -30°C before use for the next analysis

2.3. Determination of Lycopene Content

The lycopene content was determined spectrophotometrically according to Lambert-Beer equation with the specific extinction coefficient of 3450 [4]. The absorbance value at wavelength (λ) 471 nm of crude carotenoid extract in hexane was measured using UV-Vis Spectrophotometer Multispec 1700 (Shimadzu).

2.4. High Performance Liquid Chromatography (HPLC) Analysis

The analysis of crude carotenoid extract was performed by reverse-phase HPLC using a C_{30} column (YMC, 4.6 mm I.D × 150 mm, 3 μm) with a tertiary gradient elution of MeOH:MTBE:H_2O from (81:15:4, by vol.) to (6:90:4, by vol.) for 70 minutes at a flow rate of 1 mL/min according to the method of Septiany *et al.*[5].

3. Results and discussion

Separation of carotenoids from crude carotenoid extract from fresh and sweetened dried tomato is shown in Figure 1. Twelve carotenoids were separated with the dominant carotenoid detected at the retention time (t_R) 50.9 min with percentage area 88.8% (detected at maximum absorption

wavelength (λ_{max})=470 nm). Chauhan *et al.* [1] reported that lycopene is the dominant carotenoid in tomato, therefore peak 4 with λ_{max} 470 nm was lycopene. Phytoene and phytofluene as precursors for lycopene biosynthesis were detected at t_R 15.9 min (peak 1, λ_{max}=285 nm) and t_R 17.7 min (peak 2, λ_{max}=350 nm), respectively. Peak 3 at t_R 26.3 min was identified as β-carotene (λ_{max}=450 nm).

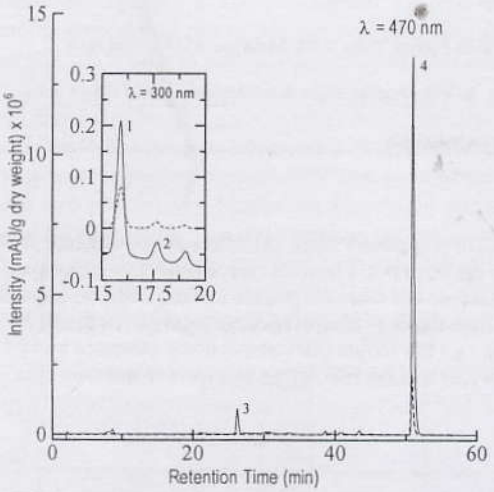

Fig 1. HPLC chromatograms of crude carotenoid extract from fresh tomato (solid line) and sweetened dried tomato (short dash) detected at λ 470 nm. Insert figure shows HPLC chromatogram detected at λ 300 nm. Peak number represents main carotenoids such as phytoene (1), phytofluene (2), β-carotene (3), All *trans* lycopene (4).

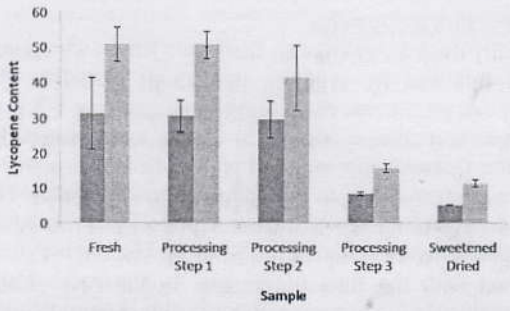

Fig 2. All *trans* isomer of lycopene content from HPLC analysis (black bar; expressed as area/100 g dry weight (dw) divided by 10^6) and total lycopene content from spectrophotometric determination (gray bar; expressed as mg/100 g dw).

Total lycopene content of fresh tomato according to the spectrophotometric method (Figure 2) was 2.84 mg/100g fresh weight (or 50.86 mg/100 g dw), in agreement with the another report from Chauhan *et al.* [1]. There is no significant change of lycopene content from fresh sample until processing step 3. The most significant decrease of lycopene content reaches 62.45% occurred at the processing step 2 to step 3. The content of lycopene was decreased approximately 77.52% from fresh tomato into the final product, while its content decreased about 68.99% from fresh tomato into step 3.

Peak area of lycopene from HHPLC analysis is shown in Figure 2. All *trans* isomer of lycopene was decreased about 83.39% from fresh tomato into the final product and from fresh tomato into step 3 decreased about 72.82%. The most significant decrease of all *trans* lycopene content occurred at the processing step 2 to step 3 (reaches 71.38%). From the processing step 3 to the final product, the all *trans* isomer of lycopene content decreased about 38.88%. The peak area of *cis* isomers of lycopene from the final product was higher than the fresh tomato. All *trans* isomer of lycopene was degraded into its *cis* isomers and then decomposed into small molecules during processing [6]. Isomerization of all *trans* and *cis* isomers of lycopene and other degradation mechanisms are generated by oxygen exposure, heat and light during the processing step 2 to step 4. The other main carotenoids, i.e. phytoene, phytofluene, and β-carotene decreased significantly almost higher than 50% in the processing step 1, whereas from the processing step 2 to step 3, the content of these main carotenoids decreased about 31.32% (phytoene), 34.36% (phytofluene) and 55.39% (β-carotene).

4. Conclusion

All *trans* isomer of lycopene was the dominant pigment of fresh tomato sample. The total lycopene content was decreased about 77.52%, whereas the area of all *trans* isomer of lycopene was decreased about 83.39% from fresh tomato into the final product. The decrease of content was also observed in other main carotenoids.

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Effect of Drying Treatments on the Composition and Concentration of Fucoxanthin and Chlorophyll *a* of Three *Sargassum* Species

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Abstract

Sargassum sp. is well known as a source of hydrocolloid and pigments. In the present preliminary study, the effect of two different drying treatments (oven and sun drying) on the pigments was evaluated and compared to the fresh one. Pigments composition from three species of *Sargassum* sp. collected from Teluk Awur beach was investigated using spectroscopic and chromatographic methods. The experimental results showed that concentration of fucoxanthin (fucox) and chlorophyll *a* (chl*a*) from the three species of *Sargassum* sp. (fresh and dried) were varied from 0.51 mg · g^{-1} to 0.94 mg · g^{-1} and from 0.47 mg · g^{-1} to 2.68 mg · g^{-1} dry weight (dw), respectively estimated by HPLC method. Oven was the best drying treatment to maintain the fucox while the sun drying was the best drying treatment to maintain chl*a*.

Keywords: Sargassum sp., fucoxanthin, chlorophyll a, drying treatments, pigments
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1. Introduction

Brown seaweed is one of the edible seaweeds and has been widely used as the major commercial source of agar, alginate, and carrageenan. Besides hydrocolloid source, recently, brown seaweed has been reported for its industrial importance of potential pigments. *Sargassum* sp. is one of the edible brown seaweed and abundant in Indonesia. In fact, *Sargassum* sp. contains high amount of fucoxanthin (fucox) and chlorophyll *a* (chl*a*) [2]. Fucox is typical pigment component of *Sargassum* sp. that gives several health benefits to humans. Fucox act as anti-cancer and antiobesity [3,4].

Many reports have been published for *Sargassum* sp. on its biochemical compounds, such as pigments and fatty acid. The growth depends on several environmental factors, such as light intensity, temperature, and nutrient levels [5]. To use *Sargassum* sp., either in fresh or dry conditions, as a raw material in pigment industry, pigment analysis is required to evaluate the effect of drying treatments on the pigment quality. In this study, we analyzed the pigment composition of the seaweed in the form of fresh seaweed and dry powders and determined the best drying treatment.

2. Material and Methods

2.1. Sample Preparation

Three *Sargassum* sp. were collected from several locations in Teluk Awur beach, Jepara, Central Java, Indonesia. The seaweeds were cleaned from any associated debris by rinsing with fresh water and then samples were put into black plastic bags and placed in cooling box during the transportation to the laboratory. Seaweeds were dried using two different treatments, *i.e.* oven (50 °C, 30 h) and sun drying (30 °C, 36 h).

2.2. Extraction of Pigments

Initially fresh *Sargassum* sp. thali were frozen with liquid N_2 and followed by grinding into small particles. The pigment extraction was carried out homogenizing 0.5 g of the sample and ethanol (EtOH) in vortex, and followed by sonication to break the seaweed cells. The crude pigment extract was separated from its residue by centrifugation. The residue was continuously extracted with the same procedure until pigments were completely extracted. The crude extract was dried with the flow of N_2 gas. In the case of dry *Sargassum* sp thali powders, pretreatment by humidification was made before the pigment extraction according to the modified method of Ishihara *et al.* [6] Pigment from dry powder (0.1 g) was extracted with the same methods as fresh sample.

2.3. Pigment Determination

Absorption spectra of crude pigment extract in acetone were recorded by a UV-1700 spectrophotometer (Shimadzu) in the range of 300 nm to 800 nm. Chromatographic analysis was performed by RP-HPLC equipped with photodiode array detector (Shimadzu). A Shim-pack VP-ODS C18 column (250L × 4.6 mm; Shimadzu) was used for pigments separation according to the method of Hegazi *et al.* [7]. Chromatographic and spectroscopic properties of the

separated pigments were used for the identification. The modified standard curve of pigments from the linear equeations was used for calculationg concentration of fucox and chl *a* in mg g^{-1} dry weight (dw) [2].

3. Results and discussion

Absorption spectra of pigment extract from fresh *Sargassum* sp. species 1 showed typical absorptions of carotenoids around 400 nm to 500 nm and also chl*a* at 431 nm and 662 nm which is partially overlapped with band of carotenoids (Fig. 1a). Absorption spectra of other fresh seaweed show similar pattern. On other hand, spectral shift from 431 nm to 412 nm was observed in dried seaweed. For the drying methods using oven showed that most of the chl*a* degraded into pheophytin. Moreover, absorption spectra of sun-dried seaweed show that the soret has two peaks in 431 nm and 412 nm suggesting initial pheophytin formation. A decrease in absorbance was observed in Qy band of chl *a* due to the influence of drying process (Fig. 1a).

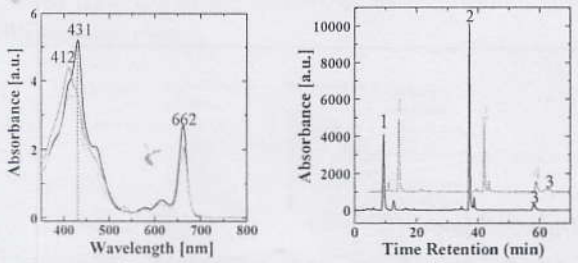

Fig. 1. (a). Absorption spectra of fresh seaweed (black), oven-dried seaweed (dark gray), and sun-dried seaweed (light gray). (b) Chromatogram of fresh (black) and dried seaweed (dark gray)

HPLC chromatogram of pigment extract from fresh and dried *Sargassum* sp. are shown in Fig. 1b. At least three main pigments were detected in fresh seaweed. On the other hand, at least four main pigments were detected in oven-dried seaweed. Morevoer, there is a slight difference on fucox intensity which appeared at t_R 9.3 min between fresh and oven-dried seaweed.

On the contrary, significant decrease was observed on chl *a* peak (t_R 37.1 min) because most of chl*a* degraded into phaeophytin. In Fig. 1b., phaeophytin is shown at t_R 53.9 min. In addition, β-carotenoid was found in t_R 58.2 min. All pigment extract had similar chromatogram profile.

The dominant pigment in fresh *Sargassum* sp. were fucox and chl*a*. The concentration of fucox varied from 0.71 mg g^{-1} to 0.87 mg g^{-1}. On other hand, chl*a* varied from 1.91 mg g^{-1} to 2.68 mg g^{-1}. From three species *Sargassum* sp. (1) has the highest concentration of fucox and chl*a*. As the result of drying treatments, fucox and chl*a* concentrations decreased (Table 1). The degradation percentage of fucox with oven drying treatment varied from 12.64 % to 29.88%, thus oven drying is recommended as the best drying method that still can maintain fucox. Meanwhile, sun drying treatment gave larger degradation percentage of fucox, *i.e.* 36.78 % to 41.38 %. The properties of carotenoids as photoprotector enable fucox to minimize degradation on chl*a* due to high intensity of light during sun drying treatment, therefore fucox concentration decreased. However, oven-dried seaweed still had stable fucox under high temperature exposure. On the other hand, chl*a* is more stable in low temperature although it was exposed under high intensity light from the sun. The decrease percentage of chl*a* from oven-dried seaweed varied from 58.58 % to 75.39 %, while the degradation percentage in sun-dried seaweed varied from 31.93 % to 56.90 %.

Table 1. Spectrophotometric and chromatographic of the chl a, total carotenoid, and fucox from *Sargassum*

Species of Sargassum	Treatment	Concentration (mg · g^{-1})	
		Chl *a*	Fucox
Sargassum sp. (1)	Fresh	2.68	0.87
	Oven	1.11	0.76
	Sun drying	1.28	0.55
Sargassum sp. (2)	Fresh	2.39	0.87
	Oven	0.68	0.61
	Sun drying	1.03	0.51
Sargassum sp. (3)	Fresh	1.91	0.71
	Oven	0.47	0.94
	Sun drying	1.30	0.61

4. Conclusion

The dominant pigments for fresh *Sargassum* sp. were fucox and chl*a*. *Sargassum* sp. (1) has the highest concentration of fucox (0.87 mg g^{-1}) and chl*a* (2.68 mg g^{-1}). The best drying treatment to maintain the concentration or quality of fucox is oven drying with decrease percentage varied from 12.64 % to 29.88 %. In other hand the best drying treatment to maintain the concentration of chl*a* is sun drying with degradation percentage varied from 31.93 % to 56.90 %.

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