



Department of Food Science
& Technology, IPB

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Mutiara Nugraheni

as

Speaker

International Seminar

**EMERGING ISSUES AND TECHNOLOGY DEVELOPMENTS
IN FOODS AND INGREDIENTS**

JAKARTA, INDONESIA, September 29th - 30th, 2010

Prof. Dr. PURWIYATNO HARIYADI, M.Sc

Chairman of
Indonesian Association of Food Technologists (IAFT)

Program & Abstracts

International Seminar EMERGING ISSUES AND TECHNOLOGY DEVELOPMENTS IN FOODS AND INGREDIENTS

Jakarta, Indonesia; September 29th - 30th, 2010



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CONTENTS

INTRODUCTION	1
ORGANIZERS	7
PROGRAM	11
SPEAKER PROFILES.....	27
ABSTRACTS	35
PARTICIPANTS.....	179

PROGRAM

DAY 1: Wednesday, September 29, 2010		
08.30 - 09.00	Registration	
09.00 - 09.05	Welcome Remarks – PATPI	
09.05 - 09.10	Welcome Remarks – Rector of IPB	
09.10 - 09.20	Opening Addressed (Head of National Agency of Drug and Food Control, Republic of Indonesia)	
09.20 - 09.30	Art Performance	
09.30 - 10.00	Keynote Speech: Recent Innovations in Packaging Technology to Ensure Safety and Quality of Food & Ingredients Prof. Dr. Gordon Robertson - <i>IUFoST and University of Queensland, Brisbane, Australia</i>	KS-1
10.00 - 10.15	Morning Break and Posters/Display Viewing	
Plenary 1. Development of Food Ingredient and Its Regulations		
10.15 - 10.45	Isoeugenol-Local Based Ingredient: Fulfill the Next Up-trend Global Demand of Natural Vanillin Thru Bioconversion Ir. Adi Hariono, MM - <i>PT Djasula Wangi, Indonesia</i>	P1-1
10.45 - 11.15	Consumer Perception on Food Safety, Food Labeling and Food Biotechnology Ms. Wisalinee Wangwittaya - <i>Asian Food Information Centre, Thailand</i>	P1-2
11.15 - 11.45	Health Claims Regulations for Functional Foods and Ingredients in ASEAN- Challenges and Opportunities Ms. Pauline Chan - <i>ILSI Southeast Asian Regions, Singapore</i>	P1-3
11.45 - 13.30	Lunch Break and Poster/ Display Viewing	
Plenary 2. Technology and Ingredient for Functional Food		
13.30 - 14.00	Benefits of Prebiotic Oligosaccharides as Ingredients for Specific Milk Formulations for Young Children Dr. Jacques G. Bindels - <i>Danone Research, Centre for Specialized Nutrition, Amsterdam, The Netherlands</i>	P2-1
14.00 - 14.30	Recent Development of Nutraceuticals Researches Relevant to Food Industry Dr. Luis Cisneros-Zevallos - <i>Department of Horticultural Science, Texas A&M University, USA</i>	P2-2
14.30 - 15.00	The effect a High Calcium Vitamin D Fortified Milk on Biochemical Markers of Bone Turnover in Postmenopausal Women- a clinical study done in Indonesia and Philippine Dr. Paulus Wijanto - <i>Eijkman-Oxford Clinical Research Unit, Indonesia</i>	P2-3
15.00 - 15.30	Afternoon Break and Posters/Display Viewing	

PROGRAM

PARALLEL SESSION			
DAY 1: Wednesday, September 29, 2010 (Cont'd)			
Parallel Session 1. Development of Local Based Food Ingredients		Parallel Session 2. Technology and Ingredients for Functional Foods	
15.30 - 15.50	PL-LS1 Lead Speaker 1a: Food Diversification and Supply Chain Management of Rural-Based Agro-processing Enterprises; Some Lesson Learned Dr. Dahruul Syah Bogor Agricultural University, Indonesia	Lead speaker 2a: Emerging Processing and Ingredients Technology Relevant to Food Industry in Indonesia Prof. Dr. Purwiyatno Hariyadi Bogor Agricultural University, Indonesia	PF-LS1 Lead Speaker 3a: Microbiological Safety of Food Ingredients Dr. Ratih Dewanti-Hariyadi Bogor Agricultural University, Indonesia
15.50 - 16.10	PL-LS2 Lead speaker 1b: Case of Food Diversification in Thailand Mrs. Darunee Edward Food Science and Technology Association of Thailand (FOSTAT) and National Center for Genetic Engineering and Biotechnology, Thailand	Lead speaker 2b: Reducing of Bitterness Compounds in Processing of Breadfruit Flour & Characterization of its Quality Dr. Sri Widowati Indonesian Center for Agricultural Postharvest Research and Development, Indonesia	Lead speaker 3b: Recent Case Studies of Food Innovation & Development in Singapore Mrs. Ser-Low Wai Ming Singapore Institute of Food Science & Technology, Singapore
16.10 - 16.25	PL-O1 Formulation of Gluten-Free Cookies Using Composite of White Sweet Potato, Rice, & Cashew Nut Sri Rejeki R. Pertiwi Djuanda University, Indonesia	Characterization of Gelatin from Pangasius Catfish (<i>Pangasius sutchi</i>) and Salimon (<i>Salmo salar</i>) Skin A.S. Babji Universiti Kebangsaan Malaysia, Malaysia	PQ-O1 Prospect of the Application of Ionizing Radiation on Mango to Extend Storage-Life and Quarantine Purposes Ridwan Rachmat Indonesian Center for Agricultural Postharvest Research and Development, Indonesia

PROGRAM

DAY 1: Wednesday, September 29, 2010 (Cont'd)

PARALLEL SESSION		Parallel Session 1. Development of Local Based Food Ingredients		Parallel Session 2. Technology and Ingredients for Functional Foods		Parallel Session 3. Quality and Safety Aspect on Food and Ingredient Development	
16.25 - 16.40	Quality Comparison of Wet Noodle from Wheat Flour with Substitution of Riau Local Corn Flour and Commercial Corn Starch <i>Shanti Fitriani</i> <i>Riau University, Indonesia</i>	PL-02	The Impact of Temperature and Heating Time Solubility of Rice Bran Healthy Drink <i>Setiarti Sukotjo</i> <i>Institute Technology Indonesia, Indonesia</i>	PF-02	Study on the Effect of Food Additives & Pasteurization to Coconut (Cocos nucifera L.) Milk Shelf Life <i>Nur Wulandari</i> <i>Bogor Agricultural University, Indonesia</i>	PQ-02	
16.40 - 16.55	Analysis of Cookies Quality from Mixed Canna Flour, Mung Bean Flour and Lentils Flour <i>Satrijo Soloko</i> <i>Mataram University, Indonesia</i>	PL-03	Rheological and Texture Properties of Acidified Dairy System as Affected by Heat Treatment and Fat Globule Size <i>Robi Andoyo</i> <i>Padjadjaran University, Indonesia</i>	PF-03	Effect of processing methods on HCN content of rubber seed (Hevea brasiliensis) <i>Daniel S. Dongoran</i> <i>Universiti Sains Malaysia, Malaysia</i>	PQ-03	
16.55 - 17.10	Sensory Property and Chemical Analysis of Snack Made from Canarium Nut <i>G.S.S. Djarkasi</i> <i>Sam Ratulangi University, Indonesia</i>	PL-04	Optimum Pasteurization Value of Pasteurized Tempe Vacuum Packed in HDPE and Aluminium Foil <i>Eko Hari Purnomo</i> <i>Bogor Agricultural University, Indonesia</i>	PF-04	Nutrient composition of Kelapa Dalam (Tall Coconut var.) and Kelapa Hibrida (Dwarf Green coconut X Tall Coconut var.) (Cocos nucifera L.) <i>Tujikha Prima Putri</i> <i>Hasanuddin University, Indonesia</i>	PQ-04	
17.10	Adjournment Day 1						
SPECIAL EVENTS							
15.30 - 17.10	GRADUATE STUDENTS RESEARCH PAPER COMPETITION						
16.00 - 18.00	MEETING ON INDONESIA QUALIFICATION FRAMEWORK FOR FOOD SCIENCE AND TECHNOLOGY						
18.30 - 21.00	PATPH'S CONGRESS (for member only)						

PROGRAM

PARALLEL SESSION				
DAY 2: Thursday, September 30, 2010				
	Parallel Session 1. Development of Local Based Food Ingredients	Parallel Session 2a. Technology and Ingredients for Functional Foods	Parallel Session 2b. Technology and Ingredients for Functional Foods	Parallel Session 3. Quality and Safety Aspect on Food and Ingredient Development
08.30 - 08.50	Lead speaker 1a: The Philippine Context of Food Manufacturing and Diversification Mr. Marty Pangamban Philippine Association of Food Technologists, Philippine	Lead speaker 2a.1: Potency of local microorganisms as functional ingredient: Probiotics Dr. Lilis Nuraida Bogor Agricultural University, Indonesia	Lead Speaker 2b.1: Characterization and Application of Carboxymethyl Cellulose Films from Pseudo Stem of Cavendish Banana (Musa cavendishii LAMBERT) Dr. Djagal W. Marseno Gadjah Mada University, Indonesia	Lead speaker 3a: Tartrazine Exposure Assessment by Using Food Frequency Method in North Jakarta Dr. Nuri Andarwulan Bogor Agricultural University, Indonesia
	PL-LS3	PF-LS3	PF-LSS	PQ-LS3
08.50 - 09.10	Lead speaker 1b: Essential Oil as Antimicrobial Food Ingredient - Case Study Basil Oil (<i>Oscinum basilicum</i>) in Agar Matrix Dr. Phan The Dong Nông Lâm University, Vietnam	Lead speaker 2a.2: Physicochemical and Functional Properties of Arrowroot, "Kimpui" and Cassava Starches Modified by Autoclaving-Freeze- Drying and Chemical treatment Prof. Dr. Betty St. Jenie Bogor Agricultural University, Indonesia	Lead speaker 2b.2: Refining and Downstreaming Processing of Crude Coconut Oil Produced by Small-Scale Industries in Bali Dr. Wayan Widia Udayana University, Indonesia	Lead speaker 3b: Management of Halal in the Malaysian Food Industry Dr. Nik Ismail Nik Daud Malaysian Institute of Food Technology (MIFT) and Universiti Kebangsaan Malaysia, Malaysia
	PL-LS4	PF-LS4	PF-LSS6	PQ-LS4

PROGRAM

DAY 2: Thursday, September 30, 2010 (Cont'd)

PARALLEL SESSION		Parallel Session 1. Development of Local Based Food Ingredients		Parallel Session 2a. Technology and Ingredients for Functional Foods		Parallel Session 2b. Technology and Ingredients for Functional Foods		Parallel Session 3. Quality and Safety Aspect on Food and Ingredient Development	
09.10 - 09.25	Chemopreventive Effect of Water Extracts Cincau Hitam (<i>Mesona palustris</i> BL) Against Interferon Gamma Expression, Immunosurveillance and Apoptosis on the Carcinogenesis of Mice Fibrosarcoma Induced by Benzo(a)pyrene Tri Dewanti Brawijaya University, Indonesia	PL-05	Isolation and Characterization of Plantaricin IR 2C12: A Novel Bacteriocin Produced by Indigenous <i>Lactobacillus Plantarum</i> 2C12 Irma Israfia Arief Bogor Agricultural University, Indonesia	PF-05	Dual Chemical Modifications to Produce an Instant Gelling Starch of Sweet Potato Sugiyono Bogor Agricultural University, Indonesia	PF-019	Antiphotoxidant Capacity of Barangan Bananas and Their Potentiality to Oxidative Stability of Oil-in-Water Emulsion Posman Sibuea Kartolik Santa Thomas University, Indonesia	PQ-05	Inhibition of Photooxidized Off Flavor in Milk with O/W Microemulsion Containing Astaxanthin and α -Tocopherol Sih Yuwanti Jember University, Indonesia
09.25 - 09.40	Chemical Properties and Glycemic Index of Cookies from Modified Arrowroot (<i>Maranta arundinaceae</i> L.) Starch Didah Nur Faridah Bogor Agricultural University, Indonesia	PL-06	Potency of Local Probiotic Base-Synbiotic Yoghurt as Functional Food Made Astawan Bogor Agricultural University, Indonesia	PF-06	Change in Aroma of Coconut Shell Liquid Smoke as Local Food Ingredient through Fractionation in Various Gradual Redistillation Temperatures Syahrani Kadir Tadulito University, Indonesia	PF-020			

PROGRAM

DAY 2: Thursday, September 30, 2010 (Cont'd)

PARALLEL SESSION		Parallel Session 1. Development of Local Based Food Ingredients		Parallel Session 2a. Technology and Ingredients for Functional Foods		Parallel Session 2b. Technology and Ingredients for Functional Foods		Parallel Session 3. Quality and Safety Aspect on Food and Ingredient Development	
09.40 - 09.55	Influence of steam Pressure and Feed Slurry Concentration on Quality of Dried Taro Porridge Processed by Drum Dryer Hadi K Purwadaria Swiss German University and Bogor Agricultural University, Indonesia	PL-07	Patency of Lactobacillus plantarum Dad 13, Inulin and its Combination to Prevent Diarrhea Caused by Pathogenic Escherichia coli in Wistar Rats Tyas Utami Gadjah Mada University, Indonesia	PF-07	Optimization of Fractionation Process of Liquid Smoke from Palm Shell using Multistage Distillation Vessel Purnama Darmadji Gadjah Mada University, Indonesia	PF-02 1	Microbiological and Sensory Quality of Wholemeal Bread with Essential Oil as Preservative Filliana Santoso Swiss German University, Indonesia	PQ-07	
09.55 - 10.30	Formulation and Frying Time Optimization of Instant Corn Noodle Dian Herawati Bogor Agricultural University, Indonesia	PL-08	Physicochemical and Functional Properties of Fermented Starch from Four Cassava Varieties Elisa Julianti Sumatera Utara University, Indonesia	PF-08	Influence of Carbon Sources and Dissolved Oxygen Concentrations (pO ₂) on Production of (1→3)- and (1→6)-glucanase by <i>Acremonium</i> sp. IMI 383268 in Batch Culture Jayus Jember State University, Indonesia	PF-02 2	Antibacterial Properties of The Essential Oil of Patchouli Alcohol (Pogostemon cablin) Yuliani Aisyah Syiah Kuala University, Indonesia	PQ-08	
10.10 - 10.30	Morning Break and Posters/ Display Viewing								

PROGRAM

DAY 2: Thursday, September 30, 2010 (Cont'd)

PARALLEL SESSION		Parallel Session 1. Development of Local Based Food Ingredients		Parallel Session 2a. Technology and Ingredients for Functional Foods		Parallel Session 2b. Technology and Ingredients for Functional Foods		Parallel Session 3. Quality and Safety Aspect on Food and Ingredient Development	
10.30 - 10.45	The Study of Added Natural Liquid Colorant from Tiwai Onion (Eleutherine americana Mierr) Extract on Kepok Banana (Musa paradisiaca Linn) Peel Jam Quality Bernatal Saragih Mulawarman University, Indonesia	PL-O9	Characterization of Edible Canna Starch (Canna edulis) and the Application as Ingredient for Some Food Products Eni Harmayani Gadjah Mada University, Indonesia	PF-O9	Taste Flavor and Compounds in Moromi (Black Soy Mash) Mary Astuti Gadjah Mada University, Indonesia	PF-O23	The Influence of Laboratory Abrasive Mill Decortication of The Local Bandung Sorghum Cultivar on The Physical and Chemical Characteristic of Decorticated Grains Carmencita Triahjadi Padjadjaran University, Indonesia	PQ-O9	Refined Gambir Extract Inhibited Lipid Oxidation in Meat Products Umar Santoso Gadjah Mada University, Indonesia
10.45 - 11.00	Optimization of Extraction and characterization of Oleoresin Nutmeg (Myristica fragrans Hoult) Origin of North Moluccas Muhammad Assagaf BPTP North Maluku, Indonesia	PL-O10	The Influence of Several Variables to the Substitution Degree Value of Esterified Tapioca Henry Herawati Indonesian Center for Agricultural Post Harvest Research And Development, Indonesia	PF-O10	Compare of Antioxidant Activity Based on Molecular Weight in Soy sauce and Model of Maillard Reaction Products Rosida DF UPN Veteran, Indonesia	PF-O24			

PROGRAM

DAY 2: Thursday, September 30, 2010 (Cont'd)

PARALLEL SESSION		Parallel Session 1. Development of Local Based Food Ingredients		Parallel Session 2a. Technology and Ingredients for Functional Foods		Parallel Session 2b. Technology and Ingredients for Functional Foods		Parallel Session 3. Quality and Safety Aspect on Food and Ingredient Development	
11.00 - 11.15	Effect of Stabilizers and Sucrose Concentration on Characteristics of Black Mulberry (<i>Morus nigra</i> L.) Juice Tantan Widiantara Pasundan University, Indonesia	PL-O11	Identification and Characterization of Cellulose from Pod Husk Cacao (<i>Theobroma cacao</i> L.) Garot S. Hutomo Taduloko University, Indonesia	PF-O11	Triterpenic Acid Content and Antioxidant Activity of Ethanolic Extract of <i>Colus tuberosus</i> Mutiara Nugraheni Yogyakarta State University, Indonesia	PF-O25	Singlet Oxygen Quenching Kinetics and Mechanism of Fucosanthin on Photosensitized Oxidation of Linoleic Acid LuFF Suhendra Udayana University, Indonesia	PQ-O11	Application of Chitosan as Natural Preservative for Prolonging the Shelf-Life of Tofu: Comparison of Incorporation and Coating Method Nugraha E. Suyatma Bogor Agricultural University, Indonesia
11.15 - 11.30	Characteristics of Maltose Syrup Produced From Enzymatic Hydrolysis of Sweet Potato Starch (<i>Ipomoea batatas</i> L.) Mira Miranti Padjadjaran University, Indonesia	PL-O12	Production of Oligosaccharides from Corn cob by Enzymatic Hydrolysis Eka Ruriani Bogor Agricultural University, Indonesia	PF-O12	Mass Transfer Modelling on Cocoa Butter Extraction Using Supercritical Fluid: Effects of Particle Size, Fermentation and pH Level of Alkalinization Asep Edi Kusnadi Pasundan University, Indonesia	PF-O26	The Taking of Oleoresin in Ginger Waste from Ginger Oil Distillation Process by Extraction Faleh Setia Budi Bogor Agricultural University, Indonesia	PQ-O12	Effect of Slice Thicknesses and Drying Methods on Quality of Dried-Slice Ginger (<i>Zingiber Officinale</i> Roscoe) Melanie Cornelia Pelita Harapan University, Indonesia
11.30 - 11.45	Effect of Variety of Citrus and Stabilizer Toward Physicochemical and Sensory Characteristic of Citrus Cloudy Juices from West Kalimantan Yohana Kusuma D Tanjungpura University, Indonesia	PL-O13	Biotechnological Production of Glucosamine as Food Ingredient Using Fungal Fermentation Cultivated in Stirred Tank Reactor Azis B. Sitanggang Pelita Harapan University, Indonesia	PF-O13		PF-O27		PQ-O13	
11.45 - 13.10	Lunch Break and Posters / Display Viewing								

PROGRAM

DAY 2: Thursday, September 30, 2010 (Cont'd)

PARALLEL SESSION		Parallel Session 1. Development of Local Based Food Ingredients		Parallel Session 2a. Technology and Ingredients for Functional Foods		Parallel Session 2b. Technology and Ingredients for Functional Foods		Parallel Session 3. Quality and Safety Aspect on Food and Ingredient Development	
13.10 - 13.25	Moisture Sorption Isotherm Properties of Instant "Ledok", a Traditional Balinese Food I Made Anom S wijaya Udayana University, Indonesia	PL-O14	Lipid Reduction and Color Properties of Spent-Duck Surimi-Like Material Using Acid-Alkaline Solubilization Methods Nurul Huda Universiti Sains Malaysia, Indonesia	PF-O14	Yield and Antibacterial Activities of Crude Ethanols Products of PKO Produced on Different Temperatures Reaction Murhadi Lampung University, Indonesia	PF-O28	Characterization of Chemical Properties of Jackfruit (<i>Artocarpus heterophyllus</i> Lamk.) Essence Powder Sri Anggrahini Gadjah Mada University, Indonesia	PQ-O14	
13.25 - 13.40	<i>Canavalia ensiformis</i> and <i>Leucaena leucocephala</i> Tempoh Fermentations Retno Indrati Gadjah Mada University, Indonesia	PL-O15	Application of Modified Starch from White Corn as a Gelatine Substitute in Formulate of Marshmallow Cream Nur Alini Soedirman University, Indonesia	PF-O15	Effect of Sugar Substitutes on Soy Yoghurt Characteristics Elaine Swiss German University, Indonesia	PF-O29	Free Amino Acids, Peptide Pattern and Cocoa Storage Protein Changes in Under-Fermented Cocoa Beans Affected by Carboxypeptidases Yusep Krowo Pasundan University, Indonesia	PQ-O15	
13.40 - 13.55	Assessment of the Usage of the Local Corn Varieties in Making Popcorn Maruli Pandjaitan Swiss German University, Indonesia	PL-O16	The Effect of Water Lily Seeds Flour and Ethyl Acetate Extract of Water Lily Seeds for Immunoglobulin A (IgA) Content in Small Intestine of Rats that Intervened Enteropathogenic <i>E. coli</i> Yusufhana Fitriah Lambung Mangkurat University, Indonesia	PF-O16	Preparation of Health Coconut Drink Using Coconut Milk, Coconut Water and Sweet Potatoes Extracts as Ingredients Netti Yuliana Lampung University, Indonesia	PF-O30	Determination of Carcinogenic Polycyclic Aromatic Hydrocarbons (PAHs) in Food by RP-HPLC-MWD Hanifah Nuryani Lioe Bogor Agricultural University, Indonesia	PQ-O16	

PROGRAM

DAY 2: Thursday, September 30, 2010 (Cont'd)

PARALLEL SESSION		Parallel Session 1. Development of Local Based Food Ingredients		Parallel Session 2a. Technology and Ingredients for Functional Foods		Parallel Session 2b. Technology and Ingredients for Functional Foods		Parallel Session 3. Quality and Safety Aspect on Food and Ingredient Development	
13.55 - 14.10	Isolation and Characterisation of Protein Isolates from Nyamplung (<i>Colophyllum inophyllum</i>) M.M. Prima Putra Gadjah Mada University, Indonesia	PL-017	Development of Liquid Surface Disinfectant using Cinnamon Essential Oil as Antibacterial Agent Hery Sutanto Swiss German University, Indonesia	PF-017	Color Stability of Jambolan (<i>Syzygium cumini</i> L. Skeels) Fruit Anthocyanins in the Model Drink Puspita Sari Jember State University, Indonesia	PF-031	The Effect of Type of Kelapa Dalam and the Length of Fermentation Time using Mould of Tempeh on the Quality of Virgin Coconut Oil Abu Amar Indonesia Institute of Technology, Indonesia	PF-017	
14.10 - 14.25	Study of Antioxidant Activity and Characteristic of Cereal Quality with Various Cooking Types on Consumer Acceptance Level Herlo Rusmarilin Sumatera Utara University, Indonesia	PL-018	Improvement of Dried Cocoa Bean Quality from Farmers by Re-fermentation Process GP Ganda Putra Udayana University, Indonesia	PF-018	The Copigmentation Effects of cinnamon Extract to the Anthocyanin Stability of Strawberry Juice Mery TD. Ambaria Pelita Horopogon University, Indonesia	PF-032	Up-regulation of Nitrite Production Induced by Maillard Product Derivatives and Activities of Natural Extracts Compounds Inhibiting Nitrite Production in Murine Neuronal Cells Anton Rahmadi Mulawarman University, Indonesia & University of Western, Australia	PF-018	
14.25 - 14.45	Break and Posters/ Display Viewing								

PROGRAM

DAY 2: Thursday, September 30, 2010 (Cont'd)	
Plenary 3. Quality and Safety Aspects on Food and Ingredient Development	
14.45 - 15.15	Emerging Issues on Potential Hazards in Food and Ingredient <i>Dr. Roland E. Poms</i> MartiCo Network of Excellence and International Cereal Center, Austria
15.15 - 15.45	Recent Technology to Support Thermal Process Validation <i>Mr. Klaus Kofod</i> Ellab A/S, Denmark
15.45 - 16.15	Sweetener Technology <i>Mr. William L. Dejer</i> The NutraSweet Company, USA
16.15 - 16.45	Development of Local Based Flour as Food Ingredient <i>Mr. Francis Welirang</i> PT Indofood Sukses Makmur Bogasari Flour Mills Tbk, Indonesia
16.45 - 17.00	Closing Remarks
SPECIAL EVENTS	
10.30 - 11.45	FIFSTA MEETING
P3-1	
P3-2	
P3-3	
P3-4	

TRITERPENIC ACID CONTENT AND ANTIOXIDANT ACTIVITY OF ETHANOLIC EXTRACT OF *COLEUS TUBEROSUS*

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ABSTRACT

Coleus tuberosus is classified as a local vegetable of family Lamiaceae, sub-family Nepetoideae and tribe Ocimeae. One characteristic of family Lamiaceae particular sub-family Nepetoideae and tribe Ocimeae is the presence of bioactive compounds triterpenic acid. Triterpenic acids have several functional properties such as antioxidants. The purpose of this study were (1) identification and determination of triterpenic acid mainly oleanolic acid (OA), and ursolic acid (UA) in peel and flesh of *Coleus tuberosus* using ethanol as solvent (2) to know the antioxidant activity of ethanolic extract of skin and flesh of *Coleus tuberosus*.

Methods for identifying kinds of triterpenic acid using HPLC. The sample was extracted with ethanol for 7 days, the solvent evaporated with waterbath and N₂ gas and then injected to HPLC, completed column of Eurosphere 100-5 C18, eluent: MeOH: 0.15% CH₃COOH (90:10) and flow rate 1 ml / min. Detector: PDA; wavelength 210 nm. Identification and determination with standar of oleanolic acid (Sigma) and ursolic acid (Santa cruz Biotech. Inc.), and antioxidant activity by DPPH method.

The results were (1) triterpenic acid of ethanolic extract of skin of *Coleus tuberosus* is higher than the flesh. ethanolic extract of peel of *Coleus tuberosus* contains OA of 19.41 µg /g sample and UA 13.61 µg/g sample. The flesh contains OA 3.64 µg/g sample and UA 3.39 µg/g sample. (2) The antioxidant activity of ethanolic extracts of the peel was higher than that of the flesh and the antioxidant activity increased with increasing concentration of the sample. The antioxidant activity of the peel at a concentration of 100 µg/ml, 200 µg/ml and 400 µg/ml : 8.7735%, 21.6038 and 60.1929%, respectively. The flesh 100 µg/ml, 200 µg/ml, and 400 µg/ml : 2.9246%, 4.6226% and 12.1689%, respectively. For the comparison, BHT 10 µg/ml, 20 µg/ml and 40 µg/ml : 20.1392%, 33.3323% and 36.4586, respectively and ascorbic acid 10 µg/ml, 20 µg/ml and 40 µg/ml, 27.7775%, 39.9305% and 91.4353%, respectively. IC₅₀ of ethanolic extract of peel of *Coleus tuberosus* was 347.82 µg/ml and IC₅₀ of the flesh was 1599.03 µg/ml. IC₅₀ BHT was 64.26 µg/ml and ascorbic acid 21.94 µg/ml. There is a positive correlation between antioxidant activity and content of triterpenic acid (OA and UA) with a correlation coefficient of 0.998.

Keyword : antioxidant, triterpenic acid, ethanol and *Coleus tuberosus*.

Introduction

The human body always exposed free radicals from outside or inside the body. Although the human body has antioxidant defenses system, but oxidative stress can be happen in case of an imbalance between the amount of antioxidants and oxidants (Reactive Oxygen Species). (Gina et al, 2009). Oxidative stress that occurs in the body can cause degenerative diseases such as diabetes mellitus, and cancer. So that the necessary efforts to increase plasma antioxidant capacity by consuming foods that have an ability as an antioxidant. *Coleus tuberosus* is one of the local vegetables of family Lamiaceae, sub-family Nepetoideae

and tribe Ocimeae. One characteristic of family Lamiaceae family, particular sub-family Nepetodeae and tribe Ocimeae is the presence of triterpenic bioactive compounds which has a function as an antioxidant. (Min Yang, 2007; Yosra et.al, 2010). Ursolic acid (UA) and oleanolic acid (OA) is a common type of triterpenic acid contained in the family Lamiaceae. Both types triterpenic acid has the chemical formula $C_{30}H_{48}O_3$. Differences in chemical structure of OA and UA influence on their functional capabilities. Although several studies have been conducted on this family, but the research related to the identification and determination of the triterpenic acid and antioxidant activity in *Coleus tuberosus* was not done. The purpose of this study were (1) identification and determination of triterpenic acid mainly oleanolic acid and ursolic acid on the peel and flesh of *Coleus tuberosus* using ethanol (2) to know the antioxidant activity of ethanolic extract of peel and flesh of *Coleus tuberosus*.

Materials and Methods

Chemicals

Ethanol, 1,1-Diphenyl-2-picryl hydrazyl (DPPH), Ascorbic acid, BHT from Sigma. Oleanolic acid (OA) standards from Sigma, Ursolic acid (UA) standard from Santa Cruz Biotech. Inc.

Plant materials

The sample was extracted with ethanol for 7 days, solvent evaporated with a waterbath and evaporated with N_2 gas. Extracts stored at $-20^{\circ}C$.

Identification and determination of oleanolic acid and ursolic acid

Identification refers to Du and Chen, (2009), the sample was extracted with ethanol (1:5) for 7 days. Filtered, evaporated and dried with waterbath and N_2 gas. The extract was dissolved methanol, filtered using $0.45 \mu M$ millex and injected into the HPLC Eurospher 100-5 C-18. Eluent: MeOH: $0.15\% CH_3COOH$ (90:10), flow $1 ml / min$, PDA detector, wavelength 210. Identification with OA and UA standards and determination using a standard curve solution with the concentration of OA and UA 12.5, 25, 50 and $100 \mu g / ml$.

Free radical scavenging activity determination

Evaluation by DPPH refers Singht et.al (2009). 2 ml of DPPH (0.1 mM in methanol), $300 \mu L$ extract of peel and flesh of *Coleus tuberosus* (100, 200 and $400 \mu g / ml$) in methanol, after 30 minutes monitoring at $\lambda 517nm$. Ascorbic acid and BHT at concentration 10, 20 and $40 \mu g / ml$ used as a control standard. Experiment three replicates. IC_{50} values for determining the concentration required to scavenging of 50% DPPH free radicals.

Result and discussion

Identification and determination of triterpenic acid (Oleanolic acid and Ursolic acid)

Identification by HPLC showed in the peel and flesh of *Coleus tuberosus*, which extracted with ethanol solvent containing triterpenic acid (OA and UA). It's like there is in Figure 1. Determination of OA using a standard curve $Y = 5154.69 x - 0.9989$ and $r^2 17960.6$. UA standard curve $Y = 9600.17 x - 45 163$ with $r^2: 0.9976$. The peel of *Coleus tuberosus* contain triterpenic acid higher than the flesh of *Coleus tuberosus*. The peel contain OA of $19.41 \mu g / g$ sample and UA: $13.61 \mu g / g$ sample. While the flesh of *Coleus tuberosus* containing OA $3.64 \mu g / g$ of sample and UA $3.39 \mu g / g$ sample.

The antioxidant activity

The antioxidant activity of ethanolic extract on the peel is higher than the flesh. The antioxidant activity increased with increasing concentration of the sample. The antioxidant activity of the skin at a concentration of $100 \mu g / ml$, $200 \mu g / ml$ and $400 \mu g / ml$: 8.7735%,

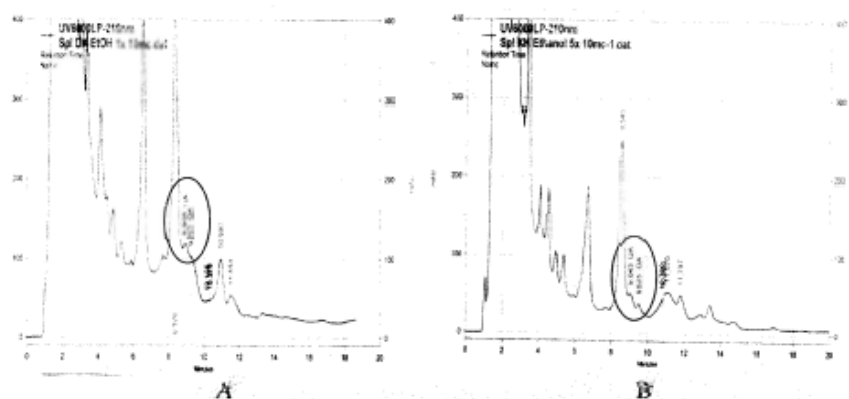


Figure 2. UA and OA ethanolic extract of flesh (A), and peel of *Coleus tuberosus* (B)

21.6038 and 60.1929, respectively. While the flesh of *Coleus tuberosus* : 100 $\mu\text{g/ml}$, 200 $\mu\text{g/ml}$ and 400 $\mu\text{g/ml}$: 2.9246%, 4.6226% and 12.1689, respectively. For the comparison, the antioxidant activity of BHT 10 $\mu\text{g/ml}$, 20 $\mu\text{g/ml}$ and 40 $\mu\text{g/ml}$: 20.1392%, 33.3323% and 36.4586, respectively. While ascorbic acid is 10 $\mu\text{g/ml}$, 20 $\mu\text{g/ml}$ and 40 $\mu\text{g/ml}$, 27.7775%, 39.9305% and 91.4353%, respectively. IC_{50} the peel of *Coleus tuberosus* is $346,98 \pm 0,32$ $\mu\text{g/ml}$ and IC_{50} the flesh of *Coleus tuberosus* is $1595,45 \pm 9,38$ $\mu\text{g/ml}$. For the comparison, IC_{50} is BHT: $64,32 \pm 1,01$ $\mu\text{g/ml}$ and ascorbic acid : 21,94 $\mu\text{g/ml}$. There is a positive correlation between antioxidant activity and content of triterpenic acid (OA and UA) with a correlation coefficient of 0.975.

Discussion

Coleus tuberosus of the family Lamiaceae, sub-family Nepetoideae and tribe Ocimeae. One characteristic is the presence of triterpenic acid compounds. In nature, more than 4000 kind of triterpene. (Patocka, 2003). However, OA and UA are often found on the family Lamiaceae. In the ethanolic extract of peel *Coleus tuberosus* (EEP) and ethanolic extract of flesh *Coleus tuberosus* (EEF) contains different OA and UA. EEP OA and UA higher than EEF. These differences in content affect of the ability as an antioxidant, it is shown on the antioxidant activity EEP was higher than EEF. This is reinforced with IC_{50} EEF less than EEP. Analysis correlation between the amount of triterpenic acid namely OA and UA with antioxidant activity is 0.975. This figure shows the positive relationship between the number of straight and berbanding OA and UA with antioxidant activity. Analysis radical scavenging activity were ascorbic acid > BHT > peel of ethanolic extract > flesh of ethanolic extract of *Coleus tuberosus*.

Conclusion

HPLC analysis of ethanolic extracts of peel and flesh of *Coleus tuberosus* show the presence of OA and UA. Analysis of antioxidants in EEP and EEF showed potential as a natural antioxidant, and the ability of these antioxidants has a correlation with content of OA and UA. The correlations showed that OA and UA is one of the bioactive compounds contain in *Coleus tuberosus*, and it have antioxidant activity. This research is expected to provide information, that *coleus tuberosus* as a vegetable is not only a source of carbohydrates but also have potential as natural antioxidants.

