



6th iSNPiNSA

International Seminar on New Paradigm and
Innovation on Natural Sciences and Its Application

**"SCIENCE AND ITS APPLICATION FOR PRODUCTIVE
AND SUSTAINABLE DEVELOPMENT"**

**Semarang, October 5-6, 2016
Grand Candi Hotel
Jl. Sisingamangaraja, Semarang, Indonesia**

PROCEEDING

**RESEARCH PAPER IN
BIOLOGY AND CHEMISTRY
Part 2 of 2 (Page: 285 – 679)**



**FACULTY OF SCIENCES AND MATHEMATICS,
DIPONEGORO UNIVERSITY
SEMARANG**



PROCEEDING

THE 6TH INTERNATIONAL SEMINAR ON NEW PARADIGM AND INNOVATION ON NATURAL SCIENCES AND ITS APPLICATION

“Science and Its Application for Productive and Sustainable
Development”

Semarang, October 5-6, 2016

Grand Candi Hotel
Jl. Sisingamangaraja, Semarang, Indonesia

Research Paper in Biology and Chemistry
Part 2 of 2 (Page: 285 – 679)

FACULTY OF SCIENCE AND MATHEMATICS
DIPONEGORO UNIVERSITY
SEMARANG



ISBN PART 2: 978-979-097-450-0

ISBN: 978-979-097-448-7

Preface

We are very pleased to introduce the proceedings of the 6th International Seminar on New Paradigm and Innovation of Natural Sciences and its Application (ISNPINSA). This 6th ISNPINSA Proceedings volume contains 107 papers derived from the written versions of most of the contributions presented during the 6th ISNPINSA. As for previous conferences, the 6th ISNPINSA is annual conferences organized by Faculty of Sciences and Mathematics (FSM), Diponegoro University (UNDIP), Semarang, Central Java, Indonesia. This seminar has been successfully conducted since 2011 and therefore becoming an annual event since then. This year, the 6th ISNPINSA was held in Grand Candi Hotel, Jl. Sisingamangaraja, Semarang, Indonesia during October 5-6, 2016.

The objectives of ISNPINSA are to facilitate brain storming and state of the art information in field of sciences and mathematics; to increase innovation of technology that can be applied in industries; to contribute in formulating strategy to increase the role of science for community; and to stimulate collaboration between industries, researchers and government to increase community welfare. The theme of 6th ISNPINSA in 2016 is **"Science and Its Application for Productive and Sustainable Development"**. This annual ISNPINSA has been intensively achieved high level improvement in Food and Energy sustainability, strengthen the collaboration between Scientists either from Indonesia or other countries, stimulated a new research partnership and contributed in formulating policies to increase the important roles of science for community.

Furthermore, this 6th ISNPINSA Proceeding is divided by two books that consisted of 107 papers. Those 107 accepted papers represented 10 groups of the research topics have been classified into 4 main fields of natural sciences, i.e. Biology (B), Chemistry (C), Mathematics (M) and Physics (P). Total accepted papers for each field of the natural sciences are as follows, Biology 52 papers, Chemistry 12 papers, Mathematics included Statistics and Computer Science 25 papers, and Physics 18 papers of which they are consisted of 10 core areas of research topics as displayed in table 1.

Table 1: The 10 Core Areas of Research Topics covered by the 6th ISNPINSA

Number	The Research Topics
1.	Nanoscience, Nanotechnology and Nanotoxicology
2.	Biotechnology for Sustainable Development and Human Welfare
3.	Biochemistry and Molecular Biology
4.	Applied Informatics and Technology, Mathematical Models
5.	Applied Ecology, Environmental Science and Sustainability
6.	Statistics for Food Security and Sustainable Agriculture
7.	Earth Science and Natural Resources Management for Environmental Sustainability
8.	Marine Biology, Aquaculture and Agriculture
9.	Integration of Chemistry Science, Technology and Engineering for the Synthesis of Multifunctional Materials and Compounds
10.	Physics and its Application for the Development of Medical Devices Technology

Last but not least, we are deeply thank you very much to the honorable keynote speakers as well as invited speakers for sharing the very interesting topic of their widely experiences



research and its application for the sustainable development of human welfare and environmental conservation in Indonesia. We are also very grateful to all participants coming from many various city and regions including from Malaysia, Papua New Guinea and Nigeria.

We also express our sincere gratitude to all advisory boards, peer review, editor administrator, and technical editor for their tremendous efforts for reviewing papers and organisation of this 6th ISNPINSA proceeding. However, we do apologize for any mistakes generated during managing the conference and organisation of the 6th ISNPINSA proceeding since preparation up to finish.

Semarang, Indonesia, August, 13th, 2017

The 6th ISNPINSA Committee

The 6th ISNPINSA Committee is pleased to announce the publication of the 6th ISNPINSA proceeding. This proceeding is the result of the 6th International Seminar on New Paradigm and Innovation of Natural Sciences and Its Application (ISNPINSA) which was held in Semarang, Indonesia, on August 13, 2017. The 6th ISNPINSA proceeding is a collection of research papers presented at the seminar. The 6th ISNPINSA proceeding is a collection of research papers presented at the seminar. The 6th ISNPINSA proceeding is a collection of research papers presented at the seminar.

The 6th ISNPINSA proceeding is a collection of research papers presented at the seminar. The 6th ISNPINSA proceeding is a collection of research papers presented at the seminar. The 6th ISNPINSA proceeding is a collection of research papers presented at the seminar.

The 6th ISNPINSA proceeding is a collection of research papers presented at the seminar. The 6th ISNPINSA proceeding is a collection of research papers presented at the seminar. The 6th ISNPINSA proceeding is a collection of research papers presented at the seminar.

1	Applied Ecology, Environmental Science and Sustainability
2	Biotechnology and Environmental Science
3	Biotechnology and Environmental Science
4	Applied and Environmental Science
5	Applied Ecology, Environmental Science and Sustainability
6	Statistics for Environmental Science and Sustainability
7	Earth Science and Natural Resources Management for Environmental Sustainability
8	Applied Ecology, Environmental Science and Sustainability
9	Statistics for Environmental Science and Sustainability
10	Physics and the Application of the Development of Modern Devices Technology

The 6th ISNPINSA proceeding is a collection of research papers presented at the seminar. The 6th ISNPINSA proceeding is a collection of research papers presented at the seminar. The 6th ISNPINSA proceeding is a collection of research papers presented at the seminar.



III. Editorial Board

- Advisory Board : Prof. Dr. Widowati, S.Si., M.Si
Drs. Sapto P. Putro, M.Si., Ph.D
Dr. Munifatul Izzati, M.Sc
- Editor in Chief : Dr. Jafron W. Hidayat, M.Sc
Vice Editor in Chief : Pratama Jujur Wibawa, Ph.D
- Peer Review of Biology : Rully Rahadian, M.Si., Ph.D (Undip)
Drs. Sapto P. Putro, M.Si., Ph.D (Undip)
Dr. Jafron W. Hidayat, M.Sc (Undip)
Dr. Hermin Pancasakti K., M.Si (Undip)
Prof. Sutrisno Anggoro (Undip)
Prof. Anthony Chesire (University of Adeleide)
Prof. Ketut Yunita, M.Sc (Udayana)
- Peer Review of Physics : Prof. Dr. Wahyu Setia Budi, MS (Undip)
Dr. Muhammad Nur, DEA (Undip)
Dr. Agus Setyawan, M.Si (Undip)
Dr. Kusworo Adi, MT (Undip)
Dr. Eng. Wisnu Jatmiko, ST., M.Kom (Undip)
Prof. Dr. Basuki (ITS)
- Peer Review of Chemistry : Adi Darmawan, Ph.D (Undip)
Pratama Jujur Wibawa, Ph.D (Undip)
Yayuk Astuti, Ph.D (Undip)
Prof. Sri Juari Santoso (UGM)
Prof. Hadi Nur (UTM)
Prof. Sugeng Tri Wahyono (UTM)
Prof. Dr. Jumina (UGM)
- Peer Review of Mathematics : Prof. Dr. Widowati, S.Si., M.Si (Undip)
Prof. Drs. Mustafid, M.Eng., Ph.D (Undip)
Farikhin, M.Sc., Ph.D (Undip)
Dr. R. Heru Tjahjana, M.Si (Undip)
Prof. Dr. Ir. Bambang Riyanto Trilaksono (ITB)
- Editorial Assistant/
Technical Editor : Alik Mauldiyah, S.Si., M.Sc (Undip)
Nadia Adlina, S.Pi., M.Si (Undip)
Gianova Andika Putri, S.Pi (Undip)
Silfiana, S.Pd (Undip)
Kenanga Sari, S.Si (Undip)
Larosi Nurfikri G. (Undip)
Nurul Lathifah (Undip)
M. Aryan Syuja' (Undip)



TABLE OF CONTENTS

	Page
Preface	II
I. Welcome Speech from Dean of FSM Undip	III
II. Welcome Speech from Chairman of the 6 th ISNPINSA	V
III. Editorial Board	VII
Research Paper in Biology	
Effect of <i>Carica papaya</i> Seeds Water Extract on Haematological Parameters of Mice Agung Janika Sitasiwi, Siti Muflichetun Mardiaty	285
Antioxidant and Antimicrobial of Carotenoid Pigment Extracted From <i>Microbacterium</i> Sp. Isolated From <i>Sargasum</i> Sp, Karimun Jawa Island Arina Tri Lunggani and Agung Suprihadi	291
Protein Content of Japanese Quail (<i>Coturnix coturnix japonica</i> L.) Egg White after Giving Turmeric Powder (<i>Curcuma longa</i> L.) On Feed Arrofi Elyas Kusumaatmaja, Silvana Tana, Tyas Rini Saraswati	295
The Differences in Phytoplankton Community Structure of Pond Waters between Pemalang and Brebes, Central Java, Indonesia Benny Diah M, Jafron W. Hidayat, Dwi E. Wibowo, Chalimah Muhammad	300
Protein Degradation and Structural Change of Calcium Oxalate in Wet Conjug Flour, Dry Conjug Flour and Conjug Soap Dewi Nur Halimah, Sri Haryanti, and Muniffatul Izzati	307
Evaluation of Applications Floating Net Cage Aquaculture Systems Integrated Multi Trophic Aquaculture (IMTA) and Monoculture an Based Growth Rate Silver Pompano (<i>Trachinotus Blochii</i>, Lacepede) Diana nasti, Sunarno ^D and Sapto P. Putro	314
The Performance of Mangrove Leaf Extract (<i>Sonneratia alba</i>) in Combating Bacterial Associated with Ice-Ice Disease of Seaweed (<i>Kappaphycus alvarezii</i>) Emmy Syafitri, Slamet Budi Prayitno, Ocky Karna Radjasa and Widodo Farid Ma'rif	319
Specific Growth Rate of Mangrove <i>Avicennia marina</i> Seedling within Silviculture Pond Canals in Semarang Coastal Area Endah Dwi Hastuti, and Rini Budihastuti	323
Molecular and Biochemical Characterization of Pink-Pigmented Thermophile Bacteria (GDG IX) from Gedong Songo Hot-Spring in Bandungan-Semarang Endang Kusdiyantini, Ahmad Edi Darmawan, Dyah Wulandari, Alfena Bagus Kusuma and Anto Budiharjo	330
Accretion of Weight Gain Various Types of Local Ducks in Central Java After Avian Influenza Vaccination Enny Yusuf Wachidah Yuniwati and Muhammad Anwar Djaelani	335
Sun Screen Protector Activity from Bacterial Symbiont Carotenoid of Soft coral <i>Sarcophyton</i> sp. Erlita Verdia Mutiara, Lia Kusmita, Nila Ayuningdiah Pratiwi, Maharani Inka Ristya Nirmala, Ocky Karna Radjasa	338
Foliar Fertilizer Application Combined with Nano-Chitosan on Growth Mangrove Seedlings <i>Rhizophora</i> Sp, <i>Brugulera</i> Sp And <i>Avicennia</i> Sp Erma Prihastanti, Sri Haryanti, Agus Subagio, Ngadiwiyana, Tony Abdillah Gumilar, Imam Ghozali, Widi Hastuti	344



Seasonal Changes on Proline Content of Leaves of Cocoa (<i>Theobroma cacao</i> L.) planted with different combinations of shade trees <i>E.Prihastanti and Y. Nurchayati</i>	354
Identification of water conservative tree species with high economic value around "Sendang Kalimah Toyyibah" <i>Erry Wiryani, Sutrisno Anggoro, and Sri Mulyani</i>	359
Contribution of Economic Sector and Agricultural Development to Support Food Security in North Sumatra Province <i>Fahmi W. Kifli and Arif Umami</i>	374
Community Structure of Macrobenthos Inhabiting Bangkong River and Fish Farming Pond Minapolitan Area of Menayu Village, Muntilan District, Indonesia <i>Fajria Darell Sofiana, Sapto P. Putro, Fuad Muhammad</i>	381
Ethnobotany of Medicinal Plants in The Vunatui Clan of The Tolai Society in East New Britain Province, Papua New Guinea <i>Felicitas Bureng, Jumari, and JafronWasiqHidayat</i>	386
Specific Immunomodulatory Activity of the Ethyl Acetate Fraction of Leaf Som Java (<i>Talinum paniculatum</i> (Jacq.) Gaertn) <i>Ika Puspitaningrum, Yuvianti Dwi Franyoto, Lia Kusmita</i>	390
The Effects of Leaves Fertilizer and Nano Silica Combination to Orchid (<i>Dendrobium</i> Sp) Growth in Subculture of Plant Tissue Culture <i>Imroetul Khasanah, Erma Prihastanti, Endah Dwi Hastuti, Agus Subagio, Ngadiwiyan</i>	396
The Optimization of Cream Formula Nanoparticles of Corncob Waste (<i>Zea mays</i> L.) Ethanol Extract Potential as Sunscreen <i>Intan Martha Cahyani, Ungsari Rizki Eka Purwanto, Tris Harni Pebriani</i>	402
Distribution and Bioaccumulation of Zn on Mangrove Coast of Tapak, Tugurejo, Semarang : An Approach to Develop Breaking Water Structure from Used Tire <i>Jafron W. Hidayat and Riche Haryati</i>	409
Biological Activities of <i>Bacillus</i> sp. from Deep Sea Sediment of Makassar Strait <i>Joko Tri Wibowo , Tutik Murniasih, Masteria Yunovilsa Putra , Alvina Nur Aini , Respati Tri Suwesono and Febriana Untari</i>	415
Analysis Amylum of Edible Plants Family Araceae in Semarang, Central Java <i>Khalisa Aini Sinaga, Murringsih, Jumari</i>	420
The Diversity Of <i>Dioscorea</i> Spp. In Central Java Indonesia: Local Utilization and Conservation <i>Jumari and Sri WidodoAgungSuedy</i>	425
Amylum's Character Of Araceae Family Edible Plants in Semarang, Central Java <i>KhalisaAiniSinaga, Murringsih, Jumari</i>	430
Ethnobotany of the Using Plant in Ceremonies of Ngadha Ethnic Jorebu'u Sub District, Ngada District, Nusa Tenggara Timur Province <i>Mariana Sada, Jumari, Rully Rahadian</i>	434
Optimization of Time Repetition and Time Echo Variation to the Image Quality in Magnetic Resonance Imaging Brain Tumor <i>Marichatul Jannah, Suryono Suryono, Gatot Murti Wibowo</i>	446



Correlation of ecological characteristics of Rice Stem Borer (Lepidoptera) with biotic and abiotic environment factors in the organic and non-organic rice fields	451
Mochamad Hadi, R.C. Hidayat Soesilohadi, F.X. Wagiman, Yayuk Rahayuningsih Suhardjono	
Community Structure and Conservation Status of Birds in Tapak Mangrove Tugurejo Semarang, Indonesia to Support Mangrove Ecotourism	455
Muhammad I. Fadila, Jafron W. Hidayat, Mochamad Hadi	
Calcium and Iron Content of Aquatic Plants From Fresh, Brackish and Marine Water Environments And Their Potency to Be Developed as Soil Conditioner	462
Munifatul Izzati	
Repetitive element palindromic PCR (rep-PCR) as a genetic tool to study diversity in Amyolytic bacteria	467
Nurhayati, Priyambada I.D., Radjasa, O.K., Widada J.	
Seahorse Bioactive Peptides Suppress Aβ24-induced Microglia Mediated Toxicity In Neuronal Cells	473
Ratih Pangestutia, and Se-Kwon Kimb	
Biodiversity of Indonesian red chilli (<i>Capsicum annum</i> var. <i>longum</i>) based on morphological characters	481
Rejeki Siti Ferniah and Sri Pujiyanto	
Daily Growth Rate of Weight, Length and Its Rasio of Milkfish (<i>Chanos chanos</i>) Within Silvicultural Pond	486
Rini Budihastuti, and Endah Dwi Hastuti	
The mycotoxin producing Aspergilli on peanut (<i>Arachis hypogea</i> L.) from traditional market In East Semarang Area	491
Ristia Rachmatunnisa & Isworo Rukmi	
Mosquito Breeding Place and Container Index are Related to Dengue Hemorrhagic Fever Cases in Uptown Semarang	496
Riza Nurul Husna, Nur Endah Wahyuningsih, Dharminto, Retno Murwani	
Potency Endophytic Mold of Rizome Temulawak (<i>Curcuma xanthorrhiza</i> Roxb) as Producer Antimicrobial toward <i>Staphylococcus aureus</i> ATCC 25923 and <i>Esherichia coli</i> ATCC 25922	502
Rokhana, Pujiyanto, Kusdiyantini	
Effort to Increase Growth of Tilapian Fish (<i>Oreochromis niloticus</i>, Linnaeus) using Different Doses of Ozonation	507
Sapto Purnomo Putro, Adhi Prasetyo, Muhammad Nur, and Zaenul Muhlisin	
Linking Chemical and Physical Parameters of A Coastal Water Ecosystem with Macrobenthic Assemblages to Assess Environmental Disturbance Caused by Fish Farming Activities	513
Sapto P. Putro, Syarif Prasetyo, Widowat ³ , Jafron W. Hidayat, and Anthony Cheshire	
The Diversity of Causative Agent Associated With Bacterial Diseases on Catfish (<i>Clarias gariepinus</i>) with Molecular Based and Their Sensitivity to Fish Drugs from Kendal, Indonesia	523
Sarjito, A. Harjuno Condro Haditomo, and Restiana W Ariyati, S. Budi Prayitno	
The Performance of Mangrove (<i>Rhizophora apiculata</i>) Leaf Extract to Treat Vibriosis (<i>Vibrio harveyi</i>) in Mud Crab (<i>Scylla serrata</i>)	530
Slamet Budi Prayitno, Sarjito, A. Mega Putri	



Purple nutsedge (<i>Cyperus rotundus</i> L.) Interference and Drought Effect on Proline Accumulation in Soybean (<i>Glycine max</i> L.) Leaves Sri Darmantli, Santosa, Laurentius Hartanto Nugroho and Kumala Dewi	
Alpha-glucosidase inhibitor activity of <i>Momordica charantia</i> L after inoculated by Endophytic bacteria Sri Pujiyanto, Sunarno, Rejeki Siti Ferniah	541
Diversity of Foreign Invasive Plants in The Forest Protected Panjang Island Jepara Central Java Sri Utami, Sutrisno Anggoro, Tri Retnaningsih Soeprubowati	545
Investigation of The Quantity Effect of NaCl Salt on Generating Glutamic Acid Contents during Shrimp Paste Production by Fermentation Method Sumardianto, Tri Winarni Agustini, and Ulfah Amalia	549
Applications Bioactive Material from Snakehead Fish (<i>Channa striata</i>) for Repair of Motor Activity and Learning-Memory Capability: A Case Study In Rats with Aging Physiological and Aging Due to Oxidative Stress Sunarno, Siti Muflichatun Mardiaty, Jatniko Endro Suseno	554
Random Amplified Polymorphic DNA - based Fingerprinting of Indonesia Medicinal Plant Ciplukan (<i>Physalis angulata</i>; Solanaceae) in Bandung, Indonesia Topik Hidayat, Didik Priyandoko, Lea Juliana Yosnata, Linda Tri Wulandari, and Siti Aisyah	561
Lake Management: Lesson Learn from Rawapening Lake Tri Retnaningsih Soeprubowati ^a	568
Identification of the <i>Bacterium</i> FJAT Secondary Metabolite by Gas Chromatography-Mass Spectrometer and Their Antimicrobial Activity Test Tutik Murniasih, Mery Maryani and Febriana Untari	574
The Resistance Monitoring Of <i>Plutella xylostella</i> (L.) Population Againsts Residual Of Emamektin Benzoate By Diagnostic Concentration Determination Udi Tarwojjo	582
Chemical and Microbiological Characterization of Katsuobushi After Boiling and Fermentation Treatment and Its Application For Flavor Enhancer Ulfah Amalia, Ima Wijayanti, and Laras Rianingsih	588
Kinetics of Growth and Production of Inulinase by fusan FF1 Wijanarka; Endang Kusdiyantini	593
<hr/> Research Paper in Chemistry <hr/>	
Nano-zeolite Modification using Cetylpyridinium Bromide for the removal of Remazol Black B and Remazol Yellow G Dyes M. Alvien Ghifari, Arofah Nuraini, Dessy Permatasari, Nur Kamilla, Teguh Imanullah, Yayuk Astuti	599
Investigation on Stability of Electroplated-Sulfurized CuInS₂-based Photocathode Modified with an In₂S₃ Layer for H₂ Evolution under Various pH Conditions Gunawan, Abdul Haris, Hendri Widiyandari, Wilman Septina, Sigeru Ikeda	606
Structural Transformation of Polystyrene Nanosphere Produce Positive and Negative Resists by Controlled Laser Exposure Jibrin Alhaji Yabagi, Mohammed Isah Kimpa, Muhammad Nmayaya Muhammad, Kasim Isah Uthaman, Embong Zaidi, and Mohd Arif Agam	614
Role Of Number Of Imprinted Cavity On The Selectivity Of The Imprinted Polymer Muhammad Cholid Djunaidi, Pardoyo	622



Chemical and Microbiological Characterization of Katsuobushi After Boiling and Fermentation Treatment and Its Application For Flavor Enhancer

Ulfah Amalia^{a*}, Ima Wijayanti^a, and Laras Rianingsih^a

^aDepartment of Fish Products Technology, Faculty of Fisheries and Marine Science, Diponegoro University, Jalan Prof. Soedarto, SH Tembalang Semarang, 50275, Indonesia

*Corresponding author Email: ulfahamalia0@gmail.com

Received on 5th September, 2016, Accepted on 13th December, 2016

This study aimed to evaluate the processing of smoked skipjack (*Euthynnus affinis*) for katsuobushi, as a based product to be a flavor enhancer for food. This study consisted two factor in the processing of katsuobushi, first the differences of fish boiling time (30 min and 60 min) and the second one was fermentation time: 1 week, 2 weeks and 3 weeks. The chemical characterization (glutamic acid, thiobarbituric acid and peroxide value) and microbiological characterization (Total Plate Count) katsuobushi were analyzed statistically using Microsoft Excel program with T-test analysis. The results showed that the total number bacteria was decreased in line with the increasing time of boiling ($p < 0.05$) and on the other hand, glutamic acid content, thiobarbituric acid and peroxide value were increased. The conclusion of this study was katsuobushi with 60 min boiling and 3 weeks fermentation was potential to be develop for fish flavor seasoning.

Keywords : katsuobushi, fish fermentation, smoked fish, glutamic acid, flavor

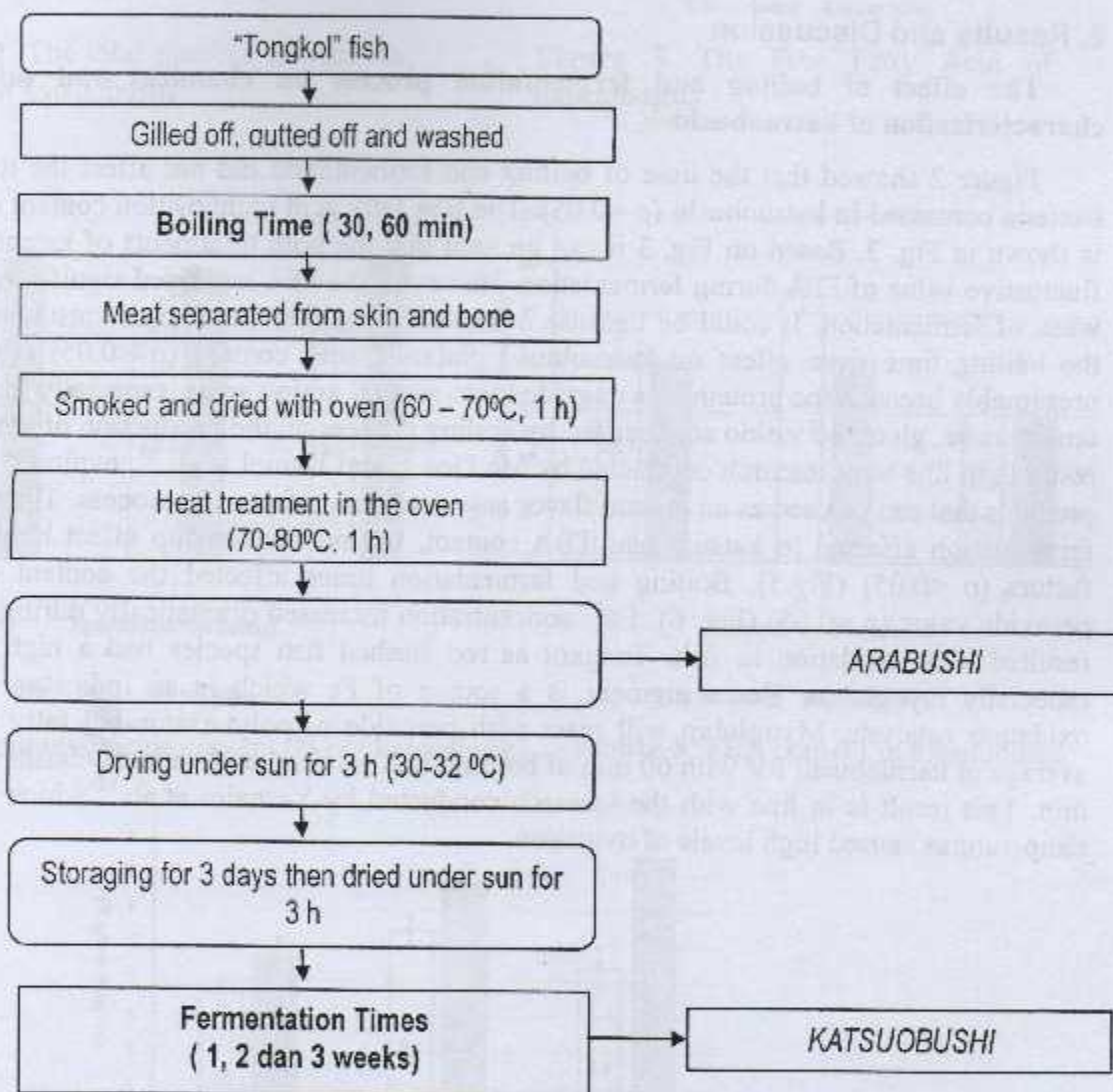
1. Introduction

Tongkol is one of marine fishes consumed and produced in Indonesia, including in Central Java. The statistical data of Marine and Fisheries Bureau, Central Java province in 2013 put the tongkol on the first position as the marine fish living in Central Java, Indonesian waters around Central Java, which potentially to be exploited and further processed. The potential value of the tongkol is about 29,000 tons per year with the 114 % utility.¹ One of the tongkol-based products that has been known is smoked tongkol. Generally, the smoking process of tongkol is performed using coconut shell, coconut husks, corn cobs, and firewood as fuel. Smoked fish products can be stored within 2-3 days at room temperature because of high water contents causing rapid microbial growth and fish spoilage.² Therefore, the innovation is needed to create smoked tongkol-based products having long-term storage, for example fish flavor (flavor). Fish flavor of fish is very popular in Japan, known as Katsuobushi, which is made from fish, bonito, with a variety of processing, among others, boiling, drying in the sun, smoking, and fermentation.³ Tongkol contains high protein of about to 23.2 grams. With the high content of protein, tongkol contains amino acids that are important for the body. Glutamic acid is one of the amino acids contained in tongkol. Glutamic acid plays a role as flavor. Normally, umami seasoning is obtained from other animal protein sources such as chicken and beef. In general, the process of making fish seasoning consist of several stages: gutting and washing the fish, cutting the fish, boiling, cooling, immersing in liquid smoke, the heating in the oven, drying in the sun, softening the texture, and fermentation. Fermentation time could be expected to affect the quality of the fish seasoning. Research related to the making fish flavor have been reported, including by Giyatmi et. al.⁴ stated that the fermentation conducted for 3 weeks was the optimum time to produce the most preferred of katsuobushi. The differences of smoking method affect the characteristic of smoked fish flavor.⁴ This study aimed to determine the effect of different boiling and fermentation time to the quality of katsuobushi produced from tongkol (*Euthynnus affinis*).⁵



2. Experimental Details

Raw materials used in this study were the fresh tongkol with weight in the range of 200-300 gram for each fish and obtained from TPI Tambak Lorok Semarang, Indonesia. The chemicals for chemical and microbiological tests were distilled water, potassium chromate, AgNO_3 , nutrient agar and others. The tools used in this study were cutting boards, pots, stoves, spinner, table drainer and digital scales. The equipments used for the analysis of the quality of katsuobushi were oven (Memmert), kjeltec system (Kjeltec 2300 Analyzer Unit; Foss Tecator AB), furnace (Memmert), soxhlet apparatus (Soxtec Avanti 2050 Auto System; Foss Tecator AB, Hoganas, Sweden), spectrophotometers (Prestige-21) and HPLC (Shimadzu RF-138). The procedure to make katsuobushi followed method proposed by Giyatmi et al⁴ with slight modifications especially in the boiling and fermentation process. The production of katsuobushi is in Fig 1.



The Fermentation

After became katsuobushi, all samples were then wrapped with thin plastic and placed in closed container plastic for 3 weeks at temperature room (30-32 °C).

Analysis of Katsuobushi

The quality analysis of katsuobushi include the total number of bacteria⁵, the content of Free Fatty Acids (FFA)⁶. 2 mL 0.5N NaOH/methanol was added to 20 mg of fat, which was later saponified for 10 minutes at 105 °C. It was examined after applying 2 mL boron trifluoride/methanol, and



methylated. Then, 2-3 mL hexane (HPLC grade) and 2 mL saturated NaCl solution were added. The supernatant of the mixture used the separated funnel was analyzed by gas chromatography (Hewlett Packard 6890 series; Palo Alto, CA, USA). The column was set up with an HP-FFAP capillary column (25 m x 0.32 mm internal diameter, 0.5 μ m film thickness); initial oven temperature of 120 $^{\circ}$ C (1 minute), increased at 2.5 $^{\circ}$ C/min to a final temperature of 230 $^{\circ}$ C (10 minutes); injector temperature 230 $^{\circ}$ C, detector temperature 250 $^{\circ}$ C; helium carrier gas with a split ratio of 20:1 and flow rate of 1 mL/min. The content of glutamic acid ⁷, Thiobarbituric Acid (TBA) ⁸, and Peroxide Value (PV) ⁹.

Data analysis

The contents of glutamic acid, TBA, PV, and the number of bacteria on katsuobushi were analyzed using ANOVA.

3. Results and Discussion

The effect of boiling and fermentation process on chemical and microbiological characterization of katsuobushi

Figure 2 showed that the time of boiling and fermentation did not affect the total number of bacteria contained in katsuobushi ($p < 0.05$). The free fatty acid composition content of katsuobushi is shown in Fig. 3. Based on Fig. 3 it can be seen that the both treatments of katsuobushi showed fluctuative value of FFA during fermentation. However, the FFA increased significantly at the third week of fermentation. It could be because of the effect of fermentation of katsuobushi. However, the boiling time gave effect on katsuobushi glutamic acid content ($p < 0.05$) (Fig. 4). This is presumably because the protein was degraded into several amino acids, especially the generation of umami taste, glutamic amino acid caused by boiling process on the production of katsuobushi. This result is in line with research conducted by Mc Gee ¹⁰ and Daniel et.al. ¹¹, explored katsuobushi as products that can be used as an umami flavor associated manufacturing process. Time of boiling and fermentation affected to katsuobushi TBA content, but no relationship effect between these two factors ($p < 0.05$) (Fig. 5). Boiling and fermentation times affected the content of katsuobushi peroxide value ($p < 0.05$) (Fig. 6). Fe²⁺ concentration increased dramatically during boiling so that resulted in at oxidation in fish. Tongkol as red fleshed fish species had a high heme pigment especially myoglobin. Heme pigment is a source of Fe which is an indicator of heating and oxidation catalysts. Myoglobin will react with peroxide of polyunsaturated fatty acids fish. The average of katsuobushi PV with 60 min of boiling time was higher than katsuobushi PV boiled in 30 min. This result is in line with the research conducted by Yusnaini et.al ¹² which stated that heat temperatures caused high levels of oxidation.



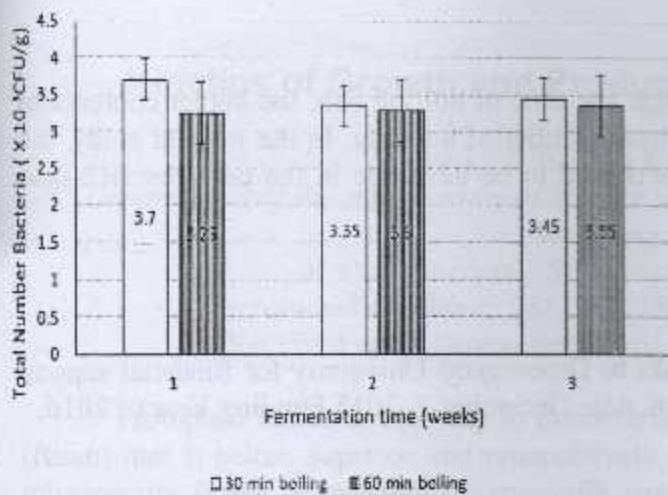


Figure 2. The total number of bacteria (cfu/g) on katsuobushi

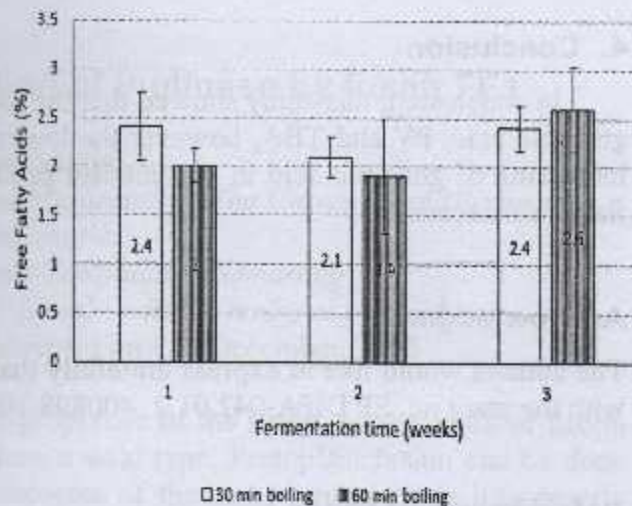


Figure 3. The Free Fatty Acid of katsuobushi

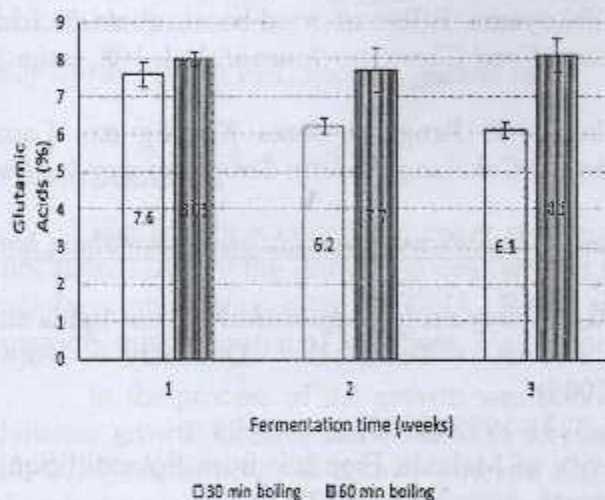


Figure 4. The glutamic acids content (%) of katsuobushi

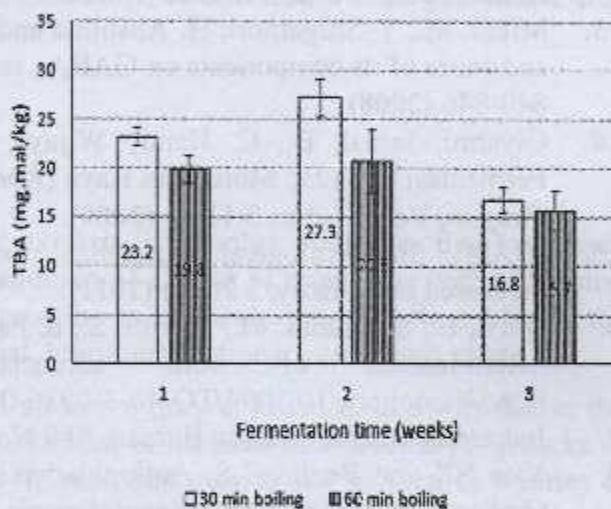


Figure 5. TBA content in katsuobushi

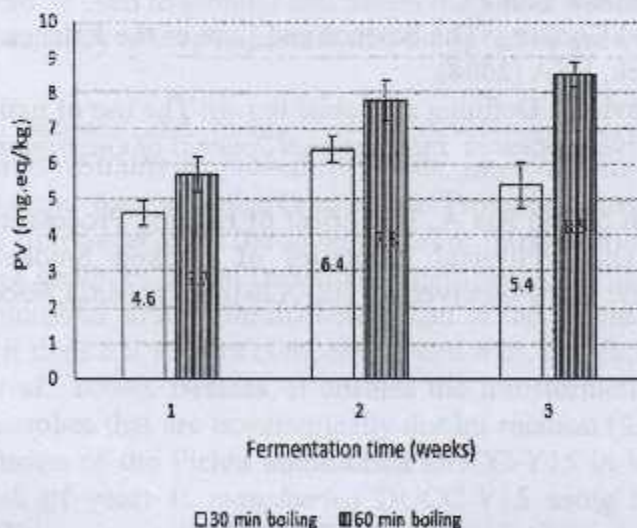


Figure 6. Peroxide value (mg.eq/kg) contained in katsuobushi

4. Conclusion

In conclusion, this study showed that the longer the time of boiling fish, the higher contents of glutamic acid, PV and TBA, however the lower total number of bacteria. In the present study, the increasing of glutamic acid in katsuobushi products tend to be developed in the next research into flavor enhancers.

Acknowledgment

The authors would like to express gratefully thanks to Diponegoro University for financial support with the grant no. SP DIPA-042.01.2. 400898/2016, date December 7, 2015 Funding Year of 2016.

References and Notes

11. [Diskanlut Jateng] Dinas Kelautan dan Perikanan Jawa Tengah. Kinerja Pelayanan Bidang Perikanan Tangkap. <http://diskanlut-jateng.go.id>. (accessed on January, 2 2016) (2016)
12. Dotulong, V. Studi Kadar Histamin Ikan Tongkol (*Auxis thazard*) Asap Yang Diawet Dengan Asam Asetat. Warta WIPTEK (Nomor : 33). ISSN : 0854-0667: 1-6 (2009)
13. Mitou, M., Y. Shigemori, H. Aoshima and S. Yokoyama. Effect of dried bonito (katsuobushi) and some of its components on GABA_A receptors. *Food Chemistry Journal*, Vol. 108, Issue 3: 840-846 (2008)
14. Giyatmi, Jamal, B., C. Hanny Wijaya, Srikandi, F. Pengaruh Jenis Kapang dan Lama Fermentasi terhadap Mutu Ikan Kayu (*Katsuobushi*) Cakalang. *Buletin Teknologi dan Industri Pangan*, Vol. XI, No. 2:11 pp (2000).
15. [BAM] Bacteriological Analytical Manual. <http://www.cfsan.fda.gov/~ebam/bam.html> (accessed on January, 2 2016) (2011).
6. Bravi, E., Sensidoni, M., Floridi, S., & Perretti, G. Fatty acids composition in beer lipids and determination of corn adjuncts. *MBAA Technical Quarterly*, 46-4, <http://dx.doi.org/10.1094/TQ-46-3-0916-01>, (2009).
7. Indonesia Standardization Bureau. SNI No. 06-3731-1995 (1995)
8. Vate NK and Benjakul S. Antioxidative Activity of Melanin Free Ink from Splendid Squid (*Loligo formosona*). *International Aquatic Research*, 5(9): 1-12 (2013).
9. AOAC . Official methods of analysis of AOAC (18th ed.) Washington, DC: Association of Official Analytical Chemists. (2007).
10. McGee, H. On Food and Cooking: The Science and Lore of the Kitchen. Scribner, New York, USA. Scribner, New York, USA (2004).
161. Daniel F., Daniel B., David C. Defining microbial terroir: The use of native fungi for the study of traditional fermentative processes. *International Journal of Gastronomy and Food Science*. Vol. 1. 64-69 (2011).
172. Yusnaini, Socparno, Edi S, and Ria A. The Effect of Heating Process using Electric and Gas Ovens on Chemical and Physical Properties of Cooked Smoked-Meat International Symposium on Food and Agro-biodiversity (ISFA2014). *Procedia Food Science* 3 : 19–20 (2015).

