Excellent Science in ASEAN

Vol. 2

Best Selected Papers and Posters from Young ASEAN Scientists on on Water-Food-Health Nexus and Information and Communication Technologies

SEA-EU-NET
partners in science

ISBN: 978-963-12-5525-6

.....

Published by:
Regional Centre for Information and Scientific Development (RCISD)
Hűvösvölgyi út 54. V/1., H-1021 Budapest
www.rcisd.eu

.....

Edited by: Ádám Dávid & Béla Kardon PhD (RCISD)
Cover design: Maria João Maia (SPI)
Printed in Vietnam by Hanoi Printing JSC
www.hanoiprinting.vn

.....

The SEA-EU-NET 2 project is funded under the 7th Framework Programme for RTD under the Capacities Programme — International Cooperation.
Grant agreement no.: 311784

www.sea-eu.net

First edition: 400 copies

CONTENTS……...

Introduction v

PART I: PAPERS ON WATER-FOOD-HEALTH NEXUS 6

THI NGOC TUYET NGUYEN, PHONG NGUYEN TAN: Water Quality of Saigon River Impacted by Ho Chi Minh Megacity Activities 6

RENATO O ARAZO: Production of High Quality Biodiesel from Fecal Sewage Sludge 12

NGUYEN HAI VAN et. al.: Antibacterial activity and mechanisms of action of essential oil extracted from *Litsea cubeba* in North Vietnam toward *Escherichia Coli* ATCC 25922 (Paper Abstract) 32

ANDRIATI NINGRUM: Pandan Leaves Extract (*Pandanous amaryllifolius Roxb.*) as Natural Antioxidant in Selected Vegetable Oils during Accelerated Storage 33

SU HLAING CHEIN et. al.: Effect of Current Post-harvest Management Practices and Identification of Fungal Species in Peanut Kernels in Myanmar 47

KHIN KHIN WIN AUNG et. al.: Estimation of Antioxidant Activity, Total Phenol and Flavonoid Content of Some Myanmar Medicinal Plants 60

TAY-ZAR AYE CHO et. al.: Qualitative Analysis of Tetracycline Residues and Evaluation of Antibiogram of Salmonella and Vibrio Isolates from Whiteleg Shrimp (*Litopenaeus Vannamei*) 70

NGO THI MAI et. al.: Acute Toxicity ff Bisphenol A Induced Phenotypic Changes on Zebrafish (*Danio Rerio*) During Early Development 79

MIMI HARYANI HASSIM: Assessing Workers Health Risk due to Fugitive Emission during Chemical Process Design Phase (Paper Abstract) 87

PART II: PAPERS ON INFORMATION AND COMMUNICATION TECHNOLOGIES 88

TATJANA TODOROVIC, ARUL CHIB: Smart Cities and the Elderly: Tensions Between Inclusion and Efficiency 88

HIEP HOANG VAN et. al.: An Evaluation of the Japanese Quasi-Zenith Satellite System LEX Augmentation Corrections in Vietnam 99

NUR AINI RAKHMAWATI et. al.: CITY113: A Mobile Application to Improve Public Participation in Surabaya City Development for Better Governance (Paper Abstract) 112

PART III: POSTERS ON WATER-FOOD-HEALTH NEXUS 113

LUU PHAM THANH et. al.: Influences of Environmental Variables on Cyanobacterial Biomass and Microcystins Concentration in a Drinking-Water Supply Reservoir, Vietnam 113

ANNA CHRISTINA R. NGO et. al.: Decolorization of Selected Synthetic Textile Dyes By Yeasts From Leaves and Fruit Peels 115

FREDERICKSON D. ENTILA et. al.: Physiological Elucidation, Genetic Architecture and Metabolic Landscape of Tolerance to Flooding during Germination in Rice.....117

SINXAY VONGPHACHANH: Comparison of Seasonal Total Water Storage Variations from Grace with Groundwater Levels, Stream Flow and Soil Moisture in Southern Laos 118

MARIA ELENA N. TANABE et. al.: Risk Assessment of Salmonella and Vibrio Cholerae Pacini in Pacific White Shrimp (Litopenaeus vannamei Boone) in Southern Philippines.....119

ANDRIATI NINGRUM: Biocatalytic Formation of Norisoprenoids as Natural Flavor from Carotenoids as Natural Colorants in Pandan Leaves (*Pandanus Amaryllifolius*) 120

PHONG T. HUYNH, PAUL TAKHISTOV: Edible Film Packaging as Nutraceutical Delivery Vehicle to Improve Human Health.....121

NAM LÊ DUY, THANG NGO-MANH: Transformation Products of Sulfamethoxazole in Aqueous Samples After 60co Gamma Irraduation 122

CHIN KOK YONG et. al.: Effects of Tocotrienol Supplementation on Biomechanical Strength of Tibia in Rats with Testosterone Deficiency.....124

PHAM VIET HUNG et. al.: Effect of Light and Hydrodynamics to Producing Spirulina Biomass in The Photobioreactor System Designed in Viet Nam 126

PART IV: POSTER ON INFORMATION AND COMMUNICATION TECHNOLOGIES 127

DR. ROLYN C. DAGUIL et. al.: Community-based ICT Infrastructure for Local Content Aggregation.....127

Introduction

SEA-EU-NET

The main aim of the first phase of the Framework Programme 7 (FP7) funded five year long SEA-EU-NET project – which started on 1 January 2008 – was to establish science, technology and innovation cooperation between the EU and ASEAN regions. One of the primary targets of the four year long second phase of the project is to stimulate deeper and more productive cooperation in three selected global societal challenges of mutual interest: Health, Food and Water. These thematic areas are of complementary interest for both regions: they account for the largest share of co-publications as well as for the joint participation in the Research and Development Framework Programmes of the European Union.

STI Days

In 2014, SEA-EU-NET 2 initiated a forum like event, the so called ASEAN-EU Science Technology and Innovation Days in order to bring together researchers, scientists, science policy makers, innovative companies and other stakeholders from ASEAN and EU countries for a yearly three-day conference on science, technology, and innovation issues. The ASEAN-EU STI Days 2016 in Hanoi are the third edition of this event, which presents academic-industry collaborations in and between ASEAN and the EU in the context of common key societal challenges for both regions.

The Publication

The book „Excellent Science in ASEAN – Best selected papers and posters from young ASEAN scientists on Water-Food-Health Nexus and Information and Communication Technologies" is the second volume of STI Days publications. It is a result of a paper and poster competition, closely linked to the ASEAN-EU STI Days 2016. The main aim of this call for papers was to provide a possibility for young ASEAN researchers to publish and to introduce their scientific research to the wide audience of the STI Days.

After the success of the first call and the first publication we have received even more applications this year. Based on the request of our sister-project CONNECT2SEA we also invited papers and posters from the field of Information and Communication Technologies (ICT). The authors of the best papers and posters were selected from more than fifty applicants, who were invited to Hanoi to take part and present their fields of research to the participants of the event. Further highly evaluated papers and poster abstracts on Water-Food-Health Nexus and ICT are available for reading in this conference paper.

PART I: PAPERS ON WATER-FOOD-HEALTH NEXUS……..

Water quality of Saigon River Impacted by Ho Chi Minh Megacity Activities

THI NGOC TUYET NGUYEN[1], TAN PHONG NGUYEN[2]

[1]Université Grenoble Alpes/IRD, France
[2]Faculty of Environment and Natural Resources, Ho Chi Minh City University of Technology, Vietnam

Abstract
Megacity in southern Vietnam is Ho Chi Minh City (HCMC), the economic capital of Vietnam, is characterized by a rapid economic, demographic, agricultural and industrial growth in the last two decades. These increases had serious consequences impact on the quality of the environment and the river crossing the city. Saigon River in Southern Vietnam represents a complex hydrological network of tributaries and distributaries that receives a large and seasonally fluctuating flow of water (flooding) and is also subjected to tidal influence. In particular, untreated urban wastewater and industrial discharges affect surface water quality. Regional issues are strong with the presence downstream of HCMC and the Saigon River of large aquaculture production areas within the protected mangrove area of Can Gio. In this study, we attempted to develop a database analysis of 6 years long (2007 – 2012) database, based on the monitoring of 8 sampling sites along Saigon River basins, we established long term evolution of the chemical state of the hydro-system and some inter-annual fluctuations of DO, COD, BOD_5, TSS, Total N and Total P. We evidenced that, beside the pristine state of the River - upstream of Ho Chi Minh City, untreated domestic and industrial wastewaters and agricultural discharges lead to the degradation of canals and Saigon River's water quality. In the downstream coastal zone, the intrusion of marine waters leads to the dilution of pollution and water quality indicators return close to its initial state.

Keywords: Saigon River, Water quality, Contamination, Nutrients

Introduction
The economic capital of Vietnam, Ho Chi Minh City with 9 million inhabitants, is one of the most dynamic metropolitan areas in South East Asia. The 10% increase of its gross domestic products and 3% increase of urban growth per year over the last ten years induced rapid economic, industrial and domestic developments. Saigon River is so important in providing the water for both domestic and industry activities in area of Ho Chi Minh City (HCMC), it is still the surface water source for Tan Hiep water treatment plan (Bao, 2011). However, in the recent years, the Saigon water quality have been nearby decreased seriously because of the discharged sources

from humans as domestic, hospital, industrial waste water, the oil leakages from aquatic traffics, landfill sites and agricultural activities (Nguyen, 2009). Among those problems, domestic waste water is the highest polluted source, many indicators excess the permit level, rising high each year as the organic excess double time, ammonium excesses eightfold to tenfold, and microorganism concentration always excess five to seven time the permission.

Saigon River spreads out across a delta of complex canals and mangrove forests under a climate regime alternating monsoon and dry periods. The high tides wash in and out of the city and a complex network of channels, culverts and sluices have been constructed to direct and control the waters. The city does not have collection networks and water treatment in line with requirements. According to the studies, less than 1% to 10% of urban and domestic water are treated before being discharged directly into canals (Marcotullio, 2007). In addition, industrial activities, with about 30 000 small industrial production plants and more than 800 large factories in 15 industrial zones, do not have adequate and effective wastewater treatment systems (Coulthart et al., 2006).

Recently, water availability has become an issue of global concern. The total freshwater resources available worldwide are estimated to be approximately 43 750 km^3/year (FAO, 2014). However, the world's water resources have decreased from 17 000 m^3/capita/year in 1950s to 7000 m^3/capita/year in the 2000s, as a result of a near doubling of the global population. Furthermore, these resources are not distributed evenly. For example, in Asia, only 3 400 m^3 of water are available/capita/year while 24 000 m^3/capita/year are available in North America. In this context, evaluating water resources and water budgets for a given region in relation to the demand of the local human population could be a key factor in optimizing water resources management and reducing environmental pollution.

Moreover in tropical areas, water quality management has become an increasingly important issue in developing countries such as Vietnam. The release of untreated wastewater into aquatic ecosystems is a common practice in many tropical countries. This practice is of great concern because the volume of wastewater produced is increasing due to rapid urbanization and economic growth (Le et al., 2014).

The objectives of this study is to evaluate the impact of megacity activities on the spatial and seasonal patterns of water quality of at 8 sampling sites along Saigon River basins based on the database of the Vietnam monitoring program of water quality assessment (2007-2012).

Materials and methods

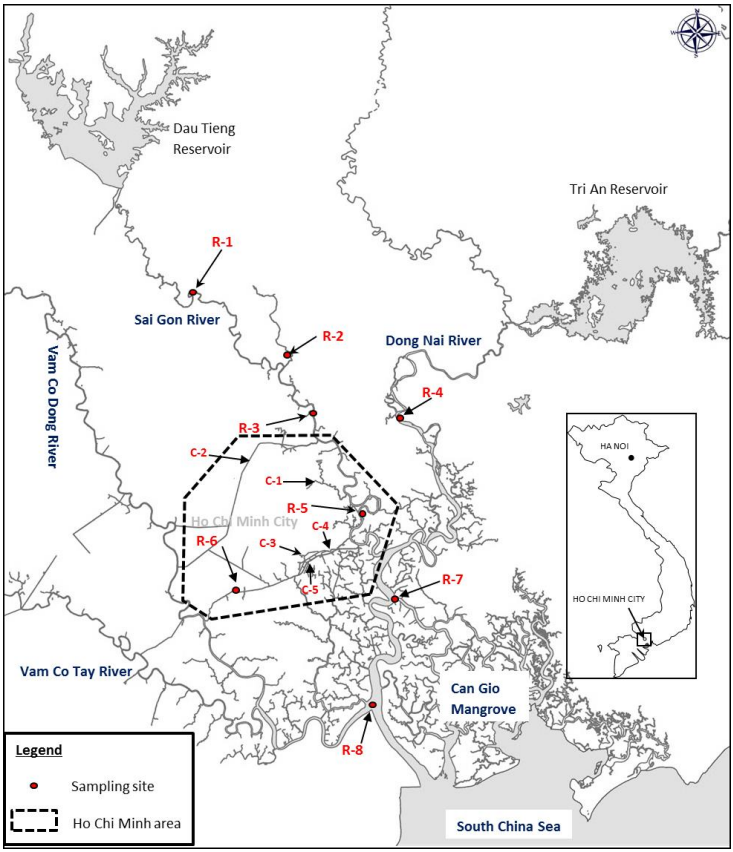
Study site

The Saigon River, located in South Vietam, is about 250km long with a catchment area of 4717 km^2 (Fig.1). It originates from Phum Daung in south-eastern Cambodia, flows to the Dau Tieng Reservoir-the fourth biggest reservoir in Vietnam (120-270km^2; 470-1680 million m^3). The regulation of its water discharges controls the intrusion of saline water downward of Phu Hoa to prevent damages on industries; especially on the water treatment plant at Phu Hoa located upstream HCMC. Downstream the reservoir, Saigon River flows through the Ho Chi Minh City where the river is connected to canals and confluences with the Dong Nai River to form the Nha Be River which flows through the Can Gio mangrove and discharges into the South China Sea, 20km north of the Mekong Delta.

This area is dominated by monsoon seasons: a wet season from May to October and a dry season from November to April. The Saigon River is affected by

asymmetric semi-diurnal tides, which induces alternating river flow direction up to 20 km downstream the Dau Tieng Reservoir and salty water intrusion upward HCMC especially during dry season. The main tributaries of the Saigon River drain wastewater from industrial zones (e.g. Thi Tinh River; Fig. 1) and domestic wastewater from city zones (e.g. urban canals; Fig. 1). The urban canals (700 km length) were built in the nineteenth century to ensure the connection of the city with the Mekong Delta, supply the city with freshwater, and control seasonal monsoon-related flood. They have been deteriorated since the last four decades due to natural and socioeconomic factors, including flood sediment deposition and a poor maintenance.

Figure 1. Saigon River Basin map and sampling sites



Database

Department of Natural Resources and Environment (DONRE) of Ho Chi Minh City (HCMC) is monitoring the daily water levels and estimated discharge measurements at a monthly basis, at 15 hydrological stations from upstream to

downstream in Saigon – Dongnai River System. For this study, we focused our analysis on 8 sampling sites along Saigon River. The notation of 8 sampling sites of monitoring is shown in Table 1. In term of water quality of urban canals and river, the database used here (DO, COD, BOD_5, TSS, Total nitrogen and Total phosphorus) was provided by DONRE. Five canals (C-1 to C-5) with ten sampling stations sampled twice in dry season and twice in wet season from 2007 to 2012 were considered. To take into account the water quality of Saigon River and its tributary, eight sampling sites (monthly sampling from 2007 to 2012) were also analysed.

Table 1: Sampling location of monitoring

Location	Code
Ben Suc	R1
Thi Tinh	R2
Phu Cuong	R3
Hoa An	R4
Phu An	R5
Binh Dien	R6
Nha Be	R7
Vam Sat	R8

Sampling sites conducted at 8 locations along Saigon River as Ben Suc, Thi Tinh, Phu Cuong, Hoa An, Phu An, Binh Dien, Nha Be, Vam Sat (station R-1 to R-8) from upstream to downstream of the Saigon River is shown in Fig.1. At each sampling site 3 samples taken at three different depths: 1m (surface water), 3m (between water layer), and 5m (lower water layer). The analysis of indicators in Saigon River sampling sites is based on the base on document of Standard Methods for Examination of Water and Wastewater (APPHA, 2005). After the analysis is completed the pollution parameters of each position are compared with the national technical standards for surface water QCVN 08: 2008 / BTNMT column A are shown in Table 2. The indicators of DO, COD, BOD_5, TSS, total N and total P are concerned to assess the impact of megacity activities on the of water quality of Saigon River.

Table 2: National technical regulations surface water quality

Parameter	DO	COD	BOD_5	TSS	Total N	Total P
Unit	(mg l^{-1})	(mg l^{-1})	(mg l^{-1})	(mg l^{-1})	(mg l^{-1})	(mg l^{-1})
QCVN 08:2008/BTNMT A1*	≥ 6	10	4	20	-	-

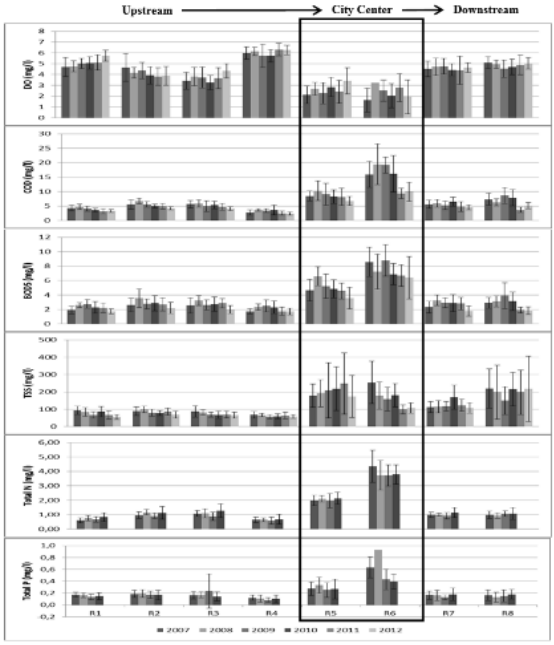
*Use for water supply purpose

Results and discussions

The upstream of Saigon River is slightly affected by local anthropogenic activities, as demonstrated by low nutrient (Total N and Total P) and TSS concentrations (Fig. 2). Besides, there is a slightly rise in Total N and Total P in Thi Tinh River – a tributary entering to Saigon River upstream sampling site R-3, which is likely related to domestic and industrial discharges from the industrial area of My Phuoc. The sampling site R-5 and R-6 located in the city centre present high levels of organic and nutrients pollution for six years with high concentration of TSS, Total N, Total P, COD, BOD_5 and low DO (<3mg/L). These contaminated conditions result from the

release of untreated wastewaters and intense anthropogenic activities which is prone to occur in many urban impacted tropical rivers.

Figure 2: Mean annual concentrations of DO, COD, BOD5, TSS, Total N and Total P measured in 8 sampling sites on the Saigon River from 2007 to 2012.



After the confluence with Dong Nai River at sampling R-4, sampling sites R-7 and R-8 reflect a typical estuarine environment with an increase of DO and a decrease of TSS, Total N, Total P, COD and BOD_5. Nutrient values decrease dramatically until reaching values similar to upstream river conditions. This trend could be explained by the dilution of the urban related effluents by seawater. In term of inter-annual fluctuations over the studied period (2007-2012), it does not present any important trends, indicating a low influence of the mean annual hydrological conditions on the level of contamination.

The Vietnam authorities are increasingly concerned about the increased risk of deterioration of water resources affecting aquatic and continental coastlines. In general, the rapid development of the economic capital of HCMC in term of urban and industrial activities is one of the most challenging issues that governmental policy and water managers have to deal within the next decade.

Conclusion

The parameters of pollutant such as DO, COD, BOD_5, TSS, total N and total P from database analysis representation for pollution of organic and nutrients indicated that water quality of Saigon River is affected by untreated wastewater from megacity activities and it characteristic is varied from upstream to downstream. The upstream

of Saigon River water quality was mainly affected by agriculture; aquaculture and forestry activities while at the centre of Saigon River where received the main source of untreated wastewater from domestic area and industrial zone discharged directly from HCMC activities. It causes the concentration excess of national technical standards for surface water QCVN 08: 2008 / BTNMT. The downstream of Saigon River is not much affected by untreated wastewater discharge from domestic and industrial activities. In addition, the water quality of the downstream was affected on the dilution of the urban related effluents by seawater.
The HCMC authorities urge to implement feasible policy to enhance awareness of people to contribute to reducing the discharge of pollutant to environment. HCMC should issue guideline, enforcement, legislation to control the untreated wastewater from domestic and industrial activities before it discharge to Saigon River.

Acknowledgment

The authors wish to thank Department of Natural Resources and Environment (DONRE) of Ho Chi Minh City (HCMC) and the laboratory staff of the Faculty of Environment and Natural Resources, Ho Chi Minh City University of Technology, for their support throughout this study.

References

APPHA., 2005. Standard Methods for Examination of Water and Wastewater, 21st Ed, American Water Works Association.
Bao, T., 2011. Study of Dynamic Mode Marine and Estuarine Water Quality Zone Saigon - Dong Nai, PhD thesis, Institute of Meteorology Hydrology and Environment, Vietnam.
Coulthart, A., Quang, N, Sharpe, H., 2006. Urban development strategy: Meeting the challenges of rapid urbanization and the transition to a market oriented economy (Monograph). Hanoi, Vietnam: World Bank.
FAO - Food and Agricultural Organization., 2014. Aquastat: Global information system on water and agriculture.
Le, T.P.Q., Billen, G., Garnier, J., Chau, V.M., 2015. Long-term biogeochemical functioning of the Red River (Vietnam): past and present Situations. Reg Environ Change. 15, 329-339.
Marcotullio P.J., 2007. Urban water-related environment transitions in Southeast Asia. Sustain Sci 2: 27-54.
Nguyen, T.V.H., 2009. Integrated Study on Factors Affecting Water Quality of the Saigon River System in Vietnam. PhD thesis, The University of Tokyo, Japan.

Production of High Quality Biodiesel from Fecal Sewage Sludge

RENATO O. ARAZO, PHD

Institute of Engineering and Technology (IET), Misamis Oriental State College of Agriculture and Technology (MOSCAT), Philippines

Abstract

The search for biofuels to augment petroleum-based fuel has led to the increased interest in finding non-food biomass as sources. In this work, the applicability of using sewage sludge feedstock for producing biodiesel via two-stage processes: fast pyrolysis and catalytic upgrading is investigated. The temperature, particle size, and vapor residence time for fast pyrolysis process were optimized. Upgrading process over a Ni/HZSM-5 catalyst was done by varying the temperature, ethanol to bio-oil mass ratio, and time. The domestic sewage sludge, largely made up of fecal waste, makes as good feedstock for producing bio-oil with high heating value of 36.43 MJ/kg, comparable to heavy fuel oil of 40 MJ/kg. Pyrolytic bio-oil major fractions were gasoline range compounds (42.17%) with undesirable oxygenated (27.0%) and nitrogenated (18.75%) compounds. Upgrading reduced the undesirable fractions resulting to bio-oil's high heating value of 39.97 MJ/kg and composed of esters (48.59%), aromatics (9.38%), alkanes (10.12%) and alkenes (5.08%).

Keywords: Bio-oil, biodiesel, catalytic upgrading, fast pyrolysis, fluidized bed, Ni/HZSM catalyst, response surface methodology, sewage sludge

Introduction

The environmental benefits of using wastes as a source of energy addresses two pressing environmental problems faced by the society today (Perla et al. 2006).

First, many entities like the wastewater treatment plant are continually challenged for the sustainable disposal of the increasing volume of sludge wastes generated from the wastewater of enlarging communities (Buys et al. 2008). For example, the EU generates more than 10 million tons (dry solids) of sewage sludge annually (Laturnus, Arnold, and Gron 2007) while South Korea recorded 2.7 million tons in 2006 (Park et al. 2010). Additionally, the stricter legislation of sludge disposal to landfill, farmland and incineration exacerbated the predicament making the wastewater managers and operators to search for a more sustainable disposal method. Several methods in breaking down the complex compounds of wastes into energy source using various technologies like thermochemical, physiochemical and biological methods hold promise in addressing wastes disposal problem (Perla et al. 2006).

Second, the imminent depletion of oil reserves around the world is becoming a major problem facing a world hungry for energy. The continuous production of primary energy from coal, natural gas and petroleum resources resulted to the incessant depletion of natural resources, the degradation of ecosystem, and the increasing amount of solid wastes, water pollution and atmospheric pollution (Perla et al. 2006). In USA alone, the consumption of primary energy is increasing at an annual average rate of 2.4%, signifying the attached increasing rates of pollution and environmental degradation. In the transportation sector, the high dependence

to energy creates volatile market with ever increasing prices of petroleum-derived fuels (Jacobson et al. 2013).

These two environmental problems, combined, bring both challenges and opportunities of recovering the value of waste for environmental and energy sustainability (Perla et al. 2006) Sludge is a special kind of biomass rich in organic compound that can potentially generate 2nd generation biofuel. The conversion of sludge wastes, a non-agricultural biomass rich in volatile combustible matter (Inguanzo et al. 2002), into bio-energy resource resolves the argument of food shortage due to competition of food and biomass production (Yin 2012; Pokoo-Aikins et al. 2010) and reduces the exploitation of agricultural lands from planting biomass sources (Bhaskar et al. 2011). More importantly, the problem of increased sludge generation from domestic wastewater treatment facilities (Laturnus, Arnold, and Gron 2007; Park et al. 2010) will be addressed. Modesty aside, this will substitute the generally unacceptable sludge routes: legislation prohibits water bodies disposal(Kelessidis and Stasinakis 2012), concentration of heavy metals, pathogens and some organic compounds raise public disagreement for composting (Fonts et al. 2012; Liu and Sun 2013), high construction costs makes land filling less attractive (Pokorna et al. 2009), and high operational cost from intensive energy requirement of incineration (Walter, Martínez, and Cala 2006; Mahmood and Elliott 2006) coupled with related costs for air pollution control (Manara and Zabaniotou 2012) and the release of toxic element like volatile organic matter makes it unacceptable in a community perspective (Domínguez et al. 2005; Pokorna et al. 2009).

Pyrolysis of waste biomass has gained interest in recent years in an attempt to recover the value of wastes through thermal decomposition in the absence of oxygen to produce biofuels such as bio-oil, biochar and biogas (Capunitan and Capareda 2012). The need for liquid biofuel in the transportation and stationary engines leads more interest in maximizing bio-oil product in many explorations (Fonts et al. 2012; Fytili and Zabaniotou 2008; Houillon and Jolliet 2005). Fast pyrolysis using fluidized bed reactor can best be done under moderate temperatures (500^{o}C), short residence time (< 2s) and rapid vapor quenching (Fonts et al. 2012; Yin 2012) resulting to the production of clean and of high quality bio-oil (Suranani and Goli 2011; Luo, Wang, and Cen 2005).

Though pyrolysis of sewage sludge has been explored in some studies (Fonts et al. 2012; Manara and Zabaniotou 2012; Pokorna et al. 2009); very limited works investigated the use of fluidized bed reactor for fast pyrolysis of digested sludge. More importantly, previous investigations focused on maximizing yield without considering other important characteristics like bio-oil's moisture content, and heating value; and the effects of process conditions. High bio-oil yield with high calorific value and low moisture content is desired. Further, the bio-oil from fast pyrolysis requires refinement generally because of low quality including but not limited to low heating value due to high oxygen fraction, high viscosity, high instability and corrosiveness (Xiu and Shahbazi 2012; L. Zhang et al. 2013; Bulushev and Ross 2011). Bio-oil from sludge, in particular, is high in nitrogen fractions which makes its application as fuel difficult (Izhar et al. 2012). To the best knowledge of the authors, no study investigated the refining of basic pH bio-oil derived from domestic wastewater sludge. Similarly, no study investigated the interactive effects of process conditions in the refinement of sludge bio-oil. Common approach in many studies, both in fast pyrolysis and refinement processes, used the conventional one-factor-at-a-time method which is generally inappropriate not only because of much cost and time needed for a large number of runs but also because the results do not account the interaction effects between parameters (Bezerra et al. 2008). These limitations, however, can be addressed by the application of response

surface methodology (RSM), a system of mathematical and statistical strategies based on the fit of a polynomial equation to experimental data (Bezerra et al. 2008). The RSM is capable of investigating multiple responses (bio-oil characteristics) over the entire variables space and to identify the region where it reaches its optimum value (Singh et al. 2011).
In this work, optimization of fast pyrolysis and catalytic upgrading processes conditions were investigated using digested sewage sludge as biomass source. Optimization of the bio-oil's yield, heating value and moisture content through fast pyrolysis process were investigated by varying reaction temperature, vapor residence time and sludge particle size. Catalytic upgrading using Ni/HZSM-5 to optimize biodiesel conversion was investigated by varying the reaction temperature, bio-oil to ethanol mass ratio and time. Particularly, this article presents the characteristics of the bio-oil from fast pyrolysis and catalytic upgrading that were produced at optimum conditions. The optimizations were determined by analyzing results of experimental runs using Design Expert 7.0 (Stat-Ease, Inc., Minneapolis, USA) software.

Materials and Methods

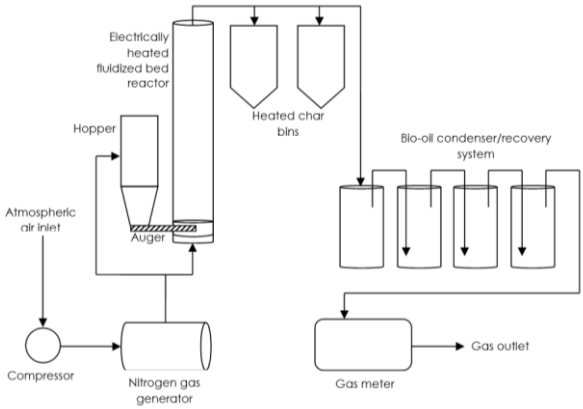
Biomass

Digested sewage sludge used in this study was collected from the Wastewater Treatment Plant of the City of College Station, Texas, USA. Sludge samples were dried in an oven at 105°C for 24h (Yuan et al. 2011), pulverized using Wiley Laboratory Mill Model#4 (distributed by Arthur Thomas Company, Philadelphia, PA, USA) and then sieved using Fischer standard brass test sieves (Fischer Scientific Company, Massachusetts USA). After sieving, powdered samples were oven-dried again for 12h (105°C) and stored in an air-tight container.

Fast pyrolysis of digested sludge

Bench-scale fluidized-bed pyrolysis reactor, developed in BETA Lab of Texas A&M University, consists of a reactor vessel, feeder system, char bin collection system, bio-oil recovery system and instrumentation (Figure 1) was used in this work. Silica sand (250-420 μm) was used to enhance heat transfer between the gas and the dried sludge. The reactor was fluidized by nitrogen gas with control valve to maintain the desired vapor residence time.

Figure1: Experimental set-up showing the fluidized bed pyrolysis reactor

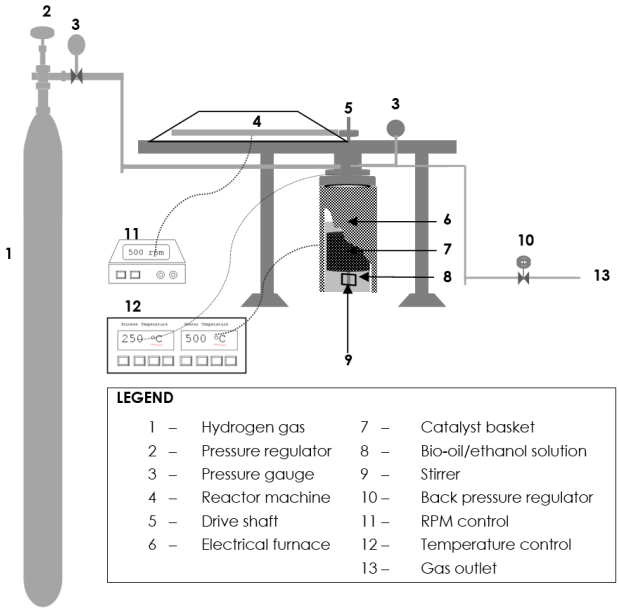


By central composite design of the RSM, 20 experimental runs were conducted varying the reaction temperature (425-550^{o}C), sludge particle size (0.2-1.0mm), and vapor residence time (1-2s). Using the results of experimental runs, Design Expert 7.0 software suggested 500^{o}C, 0.6mm, and 1.95s as best optimum conditions under the following criteria: maximize bio-oil's yield and calorific value, and minimize moisture content. Verification runs showed insignificant error which proved the validity of the suggested optimum conditions. In this work, the optimum conditions were used in the production of fast pyrolysis bio-oil for characterization.
Before starting fast pyrolysis run, the reactor was purged with nitrogen gas. Dried sludge (300g) was fed into the 20L hopper and was conveyed by a screw auger, mechanically controlled by a Movitrac LTE drive (SEW-Eurodrive Inc., Dallas, TX), into the reactor. The fast pyrolysis process took place until all volatile combustible matter of the sludge samples was vaporized.
The condensing system for bio-oil collection used four canisters connected in series and submerged in an ice/bath. To improve bio-oil collection efficiency, the 2nd and 3rd canisters were filled with 1200mL anhydrous propan-2-one (Sherwin Williams, Bryan, TX). The use of anhydrous propan-2-one as quenching agent is reported elsewhere (Guo et al. 2010).
After fast pyrolysis run, the bio-oil and bio-oil/propan-2-one mixture was collected. All canisters were washed with anhydrous propan-2-one and the mixture was added to the collected bio-oil. The propan-2-one in the bio-oil/propan-2-one mixture was removed through evaporation using Buchi Rotavapor R II (BUCHI Labortechnik AG, Switzerland) set at 60°C and vacuum pressure of 0.0556 MPa. The product yield is the ratio of the mass of the product to the mass of dry sludge introduced into the reactor, expressed in percentage.

Catalytic upgrading of bio-oil from sludge using Ni/HZSM-5 catalyst

The HZSM-5 supported Ni catalyst (Ni/HZSM-5) was prepared by wet impregnation through aqueous solution of nickel (II) nitrate hexahydrate ($Ni(NO_3)_2{\cdot}6H_2O$) over HZSM-5 support (SiO_2/Al_2O_3=27). The HZSM-5 zeolite support was provided by TRICAT (Hunt Valley, MD) while the nickel (II) nitrate hexahydrate metal precursor was purchased from Alfa Aesar (Ward Hill, MA). To impregnate 10 wt% of nickel, appropriate amount of HZSM-5 support was mixed with appropriate amount of nickel (II) nitrate hexahydrate in the solution. The support was thoroughly mixed with the metal precursor in aqueous solution, continuously stirred for 6h at 60^{o}C, and dried at 120^{o}C for 12h. The dried new Ni/HZSM-5 catalyst was calcined at 500^{o}C for 3h. Finally, it was reduced under nitrogen atmosphere for 3h at 500^{o}C.
Central composite design of RSM gave 20 experimental runs varying the temperature (150-350^{o}C), ethanol to bio-oil mass ratio (1-3 w/w), and time (2-4h). Using the results of experimental runs, Design Expert 7.0 software suggested 260^{o}C temperature, 2.5 w/w ethanol to bio-oil ratio, and 3.2h reaction time as best optimum conditions. Optimization criteria: maximize–upgraded bio-oil yield, and degrees of denitrogenation and deoxygenation. Verification runs showed insignificant error which proved the validity of the suggested optimum conditions. In this work, the optimum conditions were used in the production of catalytically refined bio-oil (upgraded bio-oil) for characterization.
A mechanically stirred 50mL Micro Robinson-Mahoney catalytic reactor (Autoclave Engineers, PA) was used in the bio-oil upgrading (Figure 2). The reactor is electrically heated by a furnace system and both have thermocouple attached. For each batch run, 1.2g Ni/HZSM-5 catalyst, 3.4g bio-oil and 8.6g ethanol were loaded; purged for 10 min with H_2 gas at 2 MPa; and then heated with H_2 gas atmosphere at 250^{o}C for 3.2h under initial pressure of 4 MPa. The mixture was rigorously stirred (~500rpm) in the entire process to eliminate diffusion limitation.

Figure 2: Micro Robinson-Mahoney catalytic reactor



As the reactor cooled at room temperature after catalytic upgrading run, aqueous and solid products were removed and separated by filtration. The bio-oil from the solid products was extracted by acetone washing. Aqueous products including the bio-oil extracted from solid products were distilled using Buchi Rotavapor R II (BUCHI Labortechnik AG, Switzerland) set at 60°C under reduced pressure of 0.0175 MPa (175 bar) to remove the unreacted solvent and the water. Similarly, the product yield is the ratio of the mass of the product to the mass of pyrolytic bio-oil used in the reaction, expressed in percentage.

Sludge biomass analyses

Moisture content of the powdered sludge was determined according to ASTM E1756. Proximate analysis of the sludge sample was performed following ASTM standards E1755 and D3172. Gross heating value (GHV) of the dried sludge and bio-oil were measured by a Parr Isoperibol Bomb Calorimeter (Model 6200, Parr Instrument Company, Moline, IL) using ASTM standard D5865. Ultimate analysis (such as C, H, N, S) of the biomass sample was performed using Vario MICRO Cube Elemental Analyzer (Elementar Analysemsysteme GmbH, Germany) following ASTM D5373.

Product analyses

Fast pyrolysis bio-oil and the subsequent upgraded bio-oil properties were determined for comparison. Heating values (HV) were determined using Parr Isoperibol Bomb Calorimeter (Model 6200, Parr Instrument Company, Moline, IL) following ASTM standard D5865. Ultimate analysis (C, H, N, S) was performed using a Vario MICRO Cube Elemental Analyzer (Elementar Analysemsysteme GmbH, Germany) following ASTM D5373. Analysis of bio-oil moisture content was determined using KF Titrino 701 (Metrohm, USA Inc.) following the ASTM E203 standard. Ash content was determined following ASTM D0482-07. Proximate and ultimate analyses follow the same procedures in the analyses of the biomass explained in the preceeding subsection.

Relative percentages of bio-oil components were determined by GC/MS using a Shimadzu QP2010Plus with a DB-5MS column (30 m x 0.25 mm i.d., 0.25 μm film thickness). A representative sample of 0.2mL was diluted in a 1mL dichloromethane. Compounds were determined using the Shimadzu GCMS Solution software (Shimadzu, Inc., Houston, TX) and NIST library. The column temperature was program according to the following sequence: 45°C for 4 min, then ramped to 250°C at 5°C/min and held at the final temperature for 10 min. Ion source temperature was set 250°C. The high purity helium gas (Airgas Inc., Charlotte, NC) served as carrier gas. The relative content of each compound was determined by peak area normalization.

Functional groups of the bio-oil were scanned using Shimadzu IRAffinity-1 FTIR (Fourier Transform Infrared) Spectrophotometer (Shimadzu, Inc., Houston, TX, USA). An attenuated total reflectance (ATR) accessory with diamond crystal was used to record bands in the region of 600–4000 cm^{-1}.

Results and Discussion

Sewage sludge characteristics

Characteristics of the digested sewage sludge sample are summarized in Table 1. The moisture content of the as received sewage sludge of 82.67 wt% was finally reduced to 1.87 wt% after oven drying at 105°C for 24 h. This is far below the fast pyrolysis recommended 10% maximum moisture content (Yin 2012) to have biomass with high organic and calorific value (Manara and Zabaniotou 2012) and less water in end products (Gil-Lalaguna et al. 2010; Westerhof et al. 2011). Compared in literature (Gao et al. 2014; J.-P. Cao et al. 2013), the percentage of volatile combustible matter of the sludge samples (68 wt%) was higher which may be attributed to the origin and composition of sewage sludge which were taken from domestic wastewater with high organic fecal wastes. In the fast pyrolysis course, the condensable volatile matter from this sludge could be collected as bio-oil.

Sludge high heating value of 18.54 MJ/kg is comparable to the as received heating values of many common biomass used for biofuel production such as peat, woodchips (softwoods), almond shells (outer), arundo donax, cotton gin trash and others (Capareda 2013). This makes the sludge an equally-potential biomass for biofuel production. Compared to many lignocellulosic materials, the higher ash content of the sludge (18%) is due to alkali metals such as Fe, Ca, K and Mg which eventually catalyze some pyrolysis reactions (Fonts et al. 2012).

Table 1. Digested sewage sludge sample characteristics

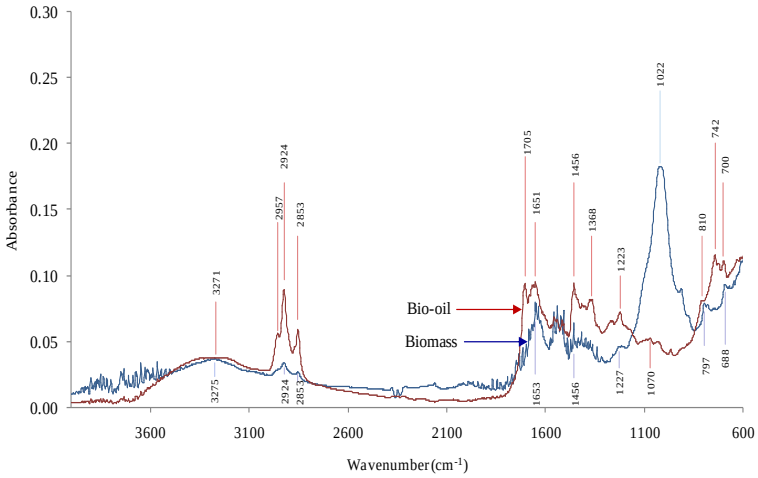
Sludge characteristics	Value		Value
Proximate analysis (%w/w)		*Ultimate Analysis (%w/w)*	
Moisture- fresh [a]	82.67±1.18	Carbon	39.45±0.70
Moisture-dry [a]	1.87±0.10	Hydrogen	5.55±0.11
Ash[b]	17.83±0.22	Oxygen [c]	28.61±1.11
Volatile matter (VM)[b]	68.07±0.57	Nitrogen	7.77±0.24
Fixed carbon [bc]	14.10±0.24	Sulfur	0.79±0.19
High heating value, MJ/kg[b]	18.54±0.15		

[a]on as received basis, [b] on dry basis, [c] determined by difference, mean ± standard error of mean (n = 3)

Result of the ultimate analysis of the sludge sample revealed percentage values within the range of those from literature (Fonts et al. 2012): carbon (23.1 – 39.9), hydrogen (3.8 – 5.9), nitrogen (2.5 – 7.9), sulfur (0.8 – 1.0), and oxygen (18.8 – 23.5). The relatively high nitrogen content can be appreciated when it is extracted for fertilizer purposes but becomes drawback when it is elevated to the liquid biofuel requiring additional processing for removal. Further, the result shows moderately low carbon content with considerable percentage of oxygen. For these reasons, sludge is more useful when converted to biofuel product with high carbon and hydrogen contents and low oxygen and nitrogen fractions.

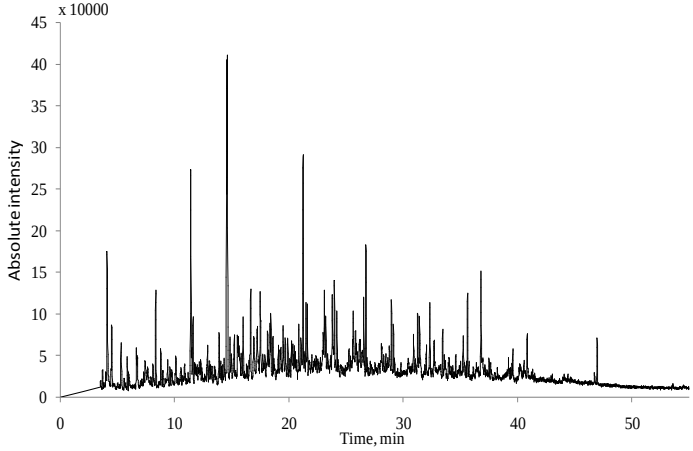
FTIR and GC-MS analyses results

Figure 3: FTIR spectra of the sludge biomass and the resulting bio-oil



In general, the FTIR spectra of the biomass and the resulting bio-oil show similar bands except the intense band of the biomass at 1022cm^{-1} (Figure 3). That peak band at 1022 cm^{-1} is attributed to OH vibration of the mineral compounds present in the sludge (Francioso et al. 2010). This disparity observed between the biomass sludge and the resulting bio-oil indicated that the high mineral content of the biomass was not incorporated in the liquid product.
A broad absorption band observed between 3500–3300 cm^{-1} is attributed to the OH stretching of hydroxyl groups from phenol, alcohol, carboxylic groups and NH stretching of amines and amide groups (Gasco, Cueto, and Méndez 2007). The presence of nitrogen compounds can also be observed in $–NO_2$ stretching and NH bending between 1550–1490 cm^{-1} (Dutta, Sarkar, and Mukherjee 2014). Using the practical approach of the published FTIR interpretation (Coates 2000), other infrared spectra were identified. The observed bands between 2900–2850 cm^{-1} corresponds to the symmetric and asymmetric stretching vibrations of aliphatic C–H bonds in the CH_3 and CH_2 groups indicating the presence of alkanes. The observed peak at 1750–1650 cm^{-1} is caused by C=O stretching vibrations suggesting the presence of ketones, aldehydes and esters. The absorption bonds observed between 1690 and 1575 cm^{-1} possibly caused by the double bonded groups like imino groups (>C=N–), azo groups (–N=N–) or the C=O of amides. Another possibility is the presence alkenes in the bonds between 1650–1580 cm^{-1}. The spectra between 1365–1470 cm^{-1} showed the presence of asymmetric and symmetric CH bending of methyl, particularly alkanes, including the gem-dimethyl and ter-butyl. The presence of CO stretching and OH bending is represented by bonds between 1300–950 cm^{-1} indicating the presence of alcohol while the bonds at frequency range between 950–650 cm^{-1} is caused by C–H in plane benching indicating the presence of aromatic compounds (Dutta, Sarkar, and Mukherjee 2014).

Figure 4: The GC-MS chromatogram of fast pyrolysis bio-oil from digested sewage sludge



Result of the FTIR spectra indicated the presence of various compounds both in the biomass and in the resulting bio-oil. These include alkanes, alkenes, ketones,

aldehydes, esters, nitrogenous compounds, alcohols, phenols, aromatic/heterocyclic and other compounds. This result confirms the upshot of the GC-MS analysis of the bio-oil (Table 5) indicating various compounds detected in the bio-oil. The complexity of the organic compounds in the pyrolysis oil is demonstrated in the GC-MS chromatogram presented in Figure 4.

Product yields

Fast pyrolysis of the digested sewage sludge from domestic wastewater resulted to relatively higher bio-oil (35.68 wt%) than biochar (23.52 wt%) and biogas (28.66 wt%) yields (Table 2). Although bio-oil yield is slightly lower than those derived from wood biomass having 50-80 wt% recovery (Butler et al. 2011), the result is within the 26.7% – 43.1% (daf) bio-oil recovery from different sewage sludges (Pokorna et al. 2009) and comparable to the 30-40% (db) recovery from second generation sources (Butler et al. 2011) including sorghum (Santos 2013), and corn stover (Capunitan and Capareda 2012). Considering the increasing volume of sludge needing sustainable disposal route, this result is a promise for new and sustainable energy source that would substitute conventional biomass sources such as wood and agriwastes in making bio-oil produced via thermochemical pathways. The lower bio-oil recovery can be compensated by sludge cost which can be taken for free, unlike other biomass requiring high cost for land and other inputs in production. For these reasons, recovering the value of sewage sludge through fast pyrolysis is promising for environmental and energy sustainability.

Similar to bio-oil produced from other biomass like wood and waste products, the fast pyrolysis bio-oil from sewage sludge is not exempted from undesirable properties such as low heating value, high fraction of oxygenated and nitrogenated compounds, unacceptable moisture content among others. To solve this, the bio-oil from sewage sludge was upgraded using 10 wt% Ni/HZSM-5 catalyst relative to feed bio-oil. Result indicated that 67.17 wt% of the fast pyrolysis bio-oil can be recovered as upgraded bio-oil after the catalytic upgrading process (Table 2). Losses is pegged at 15.19 wt% which can be attributed to the feed fast pyrolysis bio-oil moisture content (10.93 wt%), the water produced during reaction and the low boiling fraction compounds of the bio-oil which were all removed, together with the unreacted solvent, during distillation. The water was not removed in the fast pyrolysis bio-oil before upgrading not only because of additional expenses in processing but more importantly because it helps to improve the removal of oxygen fraction in the bio-oil (Fisk et al. 2009).

Table 2: Product yields of the fast pyrolysis of sludge and the catalytic upgrading of the resulting bio-oil

Product	Fast Pyrolysis of Sludge Biomass[1] [wt%]	Catalytic Upgrading of Fast Pyrolysis Bio-oil[1] [wt%]
Bio-oil	35.68±0.01	67.17±0.10
Biochar	23.52±0.40	15.13±0.53
Biogas	28.66±0.10	2.49±0.27
Losses	11.82±0.29	15.19±0.30

[1] indicates as received basis, mean ± standard error of mean (n = 3)

Fast pyrolysis process conditions: 500°C temperature, 0.6mm dried sludge particle size, 0.95s vapor residence time

Catalytic upgrading process conditions: 260°C temperature, 2.5 w/w ethanol to bio-oil mass ratio, 3.2h reaction time, 4MPa initial pressure, 500 rpm continuous stirring, 10 wt% Ni/HZSM-5 catalyst

Bio-oil properties

Properties of the upgraded bio-oil generally improved after upgrading over Ni/HZSM-5 catalyst (Table 3). The high heating value remarkably improved by 9.72%, from 36.43 MJ/kg to 39.97 MJ/kg. This result is quite impressive and is comparable to those commercial biodiesel fuels with heating value of 40.56 MJ/kg. The increase of the heating value could be explained by the 31.99% decrease of the oxygen fraction (from 17.35 to 11.80%) in the upgraded bio-oil which eventually resulted to decreased O/C ratio of 30% (from 0.20 to 0.14). The decrease of the O/C ratio is most likely due to the use of hydrogen gas that eventually removed oxygen and saturated the double bonds of the bio-oil components (Mortensen et al. 2011). In effect the fuel grade is improved by increasing the heating value.

Ash contents of the fast pyrolysis bio-oil and upgraded bio-oil are relatively low from 0.02 to 0.01%, respectively. This result is appreciably good considering that ash can cause erosion, corrosion and gumming of engines when used as fuels (Q. Cao et al. 2004). One factor that lessens ash content of the upgraded bio-oil was the filtration step of the mixture before acetone, excess ethanol and water were removed via distillation.

Elemental analysis of the catalytically upgraded bio-oil showed significant improvement with impressive increased of carbon and hydrogen while decreased of oxygen, nitrogen and sulfur fractions. The desirable carbon and hydrogen fractions were increased by 11.11 wt% and 2.67 wt%, respectively. Sulfur content of the upgraded bio-oil is minimal at 0.1 wt% fraction. Though the upgraded bio-oil have been improved in various aspects, the remaining nitrogen content of 6.76 wt%, from the 8.36 wt% in the fast pyrolysis oil, is still undesirable for fuel application. Cost efficient removal of nitrogen fraction is necessary to make this bio-oil a substitute for petroleum-based oil.

Table 3: Characteristics of fast pyrolysis bio-oil and upgraded bio-oil

Characteristics	Fast pyrolysis bio-oil	Upgraded bio-oil	FAME (Biodiesel)[2] ASTM D6751
High heating value [MJ/kg]	36.43±0.15	39.97±0.37	40.56
Ash content [wt%]	0.02±0.004	0.01+0.00	
Ultimate Analysis [wt%]			
Carbon	65.25±0.54	72.50±0.08	77
Hydrogen	8.60±0.08	8.83±0.05	12
Oxygen[1]	17.35±0.78	11.80±0.02	11
Nitrogen	8.36±0.57	6.76±0.02	
Sulfur	0.43±0.01	0.1±0.001	0.0024 max
H/C ratio	1.56±0.001	1.39±0.01	1.85
O/C ratio	0.20±0.01	0.14±0.00	0.11
Density [kg/L]	1.078±0.01	0.946±0.001	0.73
pH	6.87±0.06	9.90±0.06	-

[1]determined by difference, %O = 100—C—H—N—S—Ash, mean ± standard error of mean (n = 3)

[2](NREL 2009)following ASTM D6751 for biodiesel (B100)

Fast pyrolysis process conditions: 500°C temperature, 0.6mm dried sludge particle size, 0.95s vapor residence time

Catalytic upgrading process conditions: 260°C temperature, 2.5 w/w ethanol to bio-oil mass ratio, 3.2h reaction time, 4MPa initial pressure, 500 rpm continuous stirring, 10wt% Ni/HZSM-5 catalyst

Densities of fast pyrolysis and upgraded bio-oil were 1.078 and 0.946 kg/L, respectively. This means that 340 L of fast pyrolysis bio-oil can be obtained from each ton of dried sludge biomass. Subsequently, the catalytic upgrading process could produce 240 L of upgraded bio-oil in every ton of dried sludge.

Contrary to most acidic bio-oil produced in fast pyrolysis, the bio-oil from digested sewage sludge is nearly neutral (pH=6.9). Catalytic upgrading resulted to as alkaline pH of 9.9. This non-acidic property was confirmed considering the minimal fraction of acid compounds detected in the GC-MS analysis (Table 4). This result is valuable for transportation and engine applications, resolving the problem of corrosion in vessels and pipeworks experienced when acidic bio-oil is used.

Bio-oil Compositions and Proposed Mechanisms in Catalytic Upgrading Result

The improved bio-oil largely composed desirable ester (48.59%) with abundant octadecanoic acid ethyl ester (12.86%). Table 4 showed comparison of the fast pyrolysis bio-oil and upgraded bio-oil compositions while Table listed the compounds detected by GC-MS analysis of the resulting bio-oil after upgrading.

GC-MS analysis determined the changes of the percentage relative contents of the compounds before and after catalytic upgrading. After upgrading, alkane fraction was abundant in the control runs indicating that its formation was neither affected by the Ni/HZSM-5 catalyst nor the ethanol co-feed. Direct hydrogenation of hydrocarbon favors the formation of these alkane compounds which most likely due to the reduction of alkene groups (Fig. 5a).

Table 4. Relative percentages of compounds detected in the fast pyrolysis bio-oil and upgraded bio-oil

Compounds	Fast pyrolysis bio-oil [relative %]	Catalytic Upgrading result[relative %]		
		Upgraded bio-oil (bio-oil+ catalyst +ethanol)	Control (bio-oil+ethanol)	Control (bio-oil+catalyst)
Acid	3.15	-	0.94	0.93
Alcohol	4.65	3.25	0.36	0.36
Aldehydes	1.74	-	-	-
Alkanes	20.00	10.12	37.35	38.17
Alkenes	19.71	5.08	4.85	4.87
Aromatics	2.46	9.38	6.74	6.71
Esters	0.88	48.59	0.72	-
Ketones	11.72	2.52	1.90	1.91
Nitrogenated compounds	18.75	14.11	35.28	35.03
Phenols	15.28	6.95	11.86	12.02
Others	1.66	-	-	-

Fast pyrolysis process conditions: 500°C temperature, 0.6mm dried sludge particle size, 0.95s vapor residence time

Catalytic upgrading process conditions: 260°C temperature, 2.5w/w ethanol to bio-oil mass ratio, 3.2h reaction time, 4MPa initial pressure, 500 rpm continuous stirring

The same trend was observed in the upgrading of energy sorghum-derived bio-oil over Pt/HZSM-5 catalyst and ethanol (Santos 2013) which demonstrated more aliphatic (alkanes) hydrocarbon observed in control run (without catalyst). Further, the addition of hydrogen atoms in the C=C bond is known to convert alkenes to alkanes (Research & Education Association 2007). This means that abundance of alkanes in the control runs is generally be attributed to hydrogenation of alkenes into alkanes. Conversely, the undesirable formation of nitrogen-containing compounds also increased in control runs demonstrating that its formation is dependent on thermal reaction without significant effect of neither Ni/HZSM-5 catalyst nor the ethanol.

Phenols content showed 55% reduction (from 15.28% to 6.95%). This result indicated that phenol compounds likely proceed direct hydrolysis converting it to benzene and water or through direct hydrogenation producing cyclohexane (Jacobson et al. 2013). This mechanism is also proposed in other studies (Massoth et al. 2006; Yunquan et al. 2008). Investigation using model phenol upgrading showed conversion from 9.3 wt% using HZSM-5 alone at 370^{o}C (Adjaye and Bakhshi 1995) to 91.8% using HZSM-5 (Si/Al=38) loaded with 10% Ni (X. Zhang et al. 2013). Unlike model compound upgrading, this work used actual bio-oil in which complex reaction might took place simultaneously in the process. In this effect, a mechanism in the phenol conversion is proposed (Fig. 5b). However, the cyclohexane from phenol may still proceed another reaction following other proposed mechanism.

Ketone compounds significantly decreased from 11.72% to 2.52%. It indicated incidental hydrogenation/dehydration of ketones giving up oxygen more easily in the formation or breakdown of double bonds where the oxygen was attached (Jacobson et al. 2013). Most likely, reduction of ketones to alcohol by hydrogenation took place. The resulting alcohol reacted the carboxylic acid (produced from nitrile hydrolysis) and Ni/HZSM-5 which eventually produced esters (Fig. 5d). This mechanism is also proposed in other work (Research & Education Association 2007) and is adapted here as practically applicable considering the abundance of esters in the improved bio-oil.

It is by surprised that the upgraded bio-oil composed largely of esters (48.59%) making its mechanisms difficult to trace because fast pyrolysis bio-oil as feedstock did not contain much acids. Ester production supposedly proceeds through conversion of readily available acid compounds via esterification/transesterification process. With this, possible pathways/mechanisms in the conversion of alkanes, alkenes and nitrogenated compounds are proposed (Fig. 5a and 5f). With nickel base catalyst in the HZSM-5, the considerable amount of nitrile-containing compounds in the feed bio-oil may be hydrolyzed which resulted to the complimentary release of nitrogen from the bio-oil via ammonia gas (Research & Education Association 2007) and the subsequent conversion to carboxylic acids. The presence of ethanol furthers the reaction by converting carboxylic acids into desirable ester products and water via esterification (Fig. 5a).

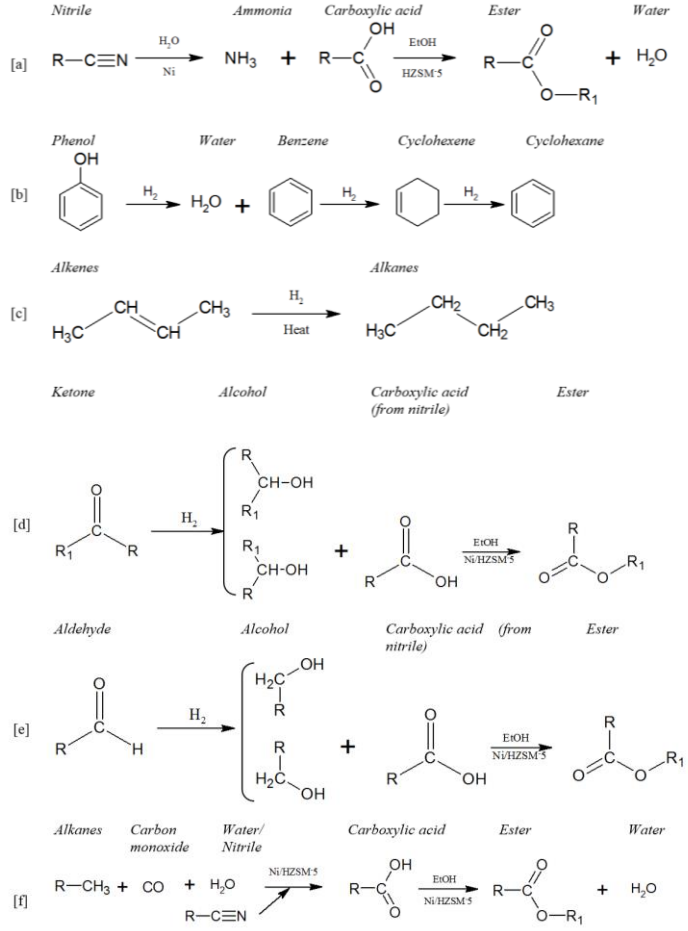
Table 5. Components of the upgraded bio-oil detected by the GC-MS

Compounds	% Relative Content	Compounds	% Relative Content
Alcohols	**3.25**	Butanoic acid, 2-methyl-, ethyl ester	0.3
1-Hexanol, 2-ethyl-	0.91	Butanoic acid, 3-methyl-, ethyl ester	0.79
Benzyl alcohol, 3-ethylamino-	0.67	Pentanoic acid, ethyl ester	0.45
1-Hexadecanol	1.04	Hexanoic acid, ethyl ester	1.1
1-Eicosanol	0.63	Pentanoic acid, 4-methyl-, ethyl ester	0.99
Alkanes	**10.12**	Heptanoic acid, ethyl ester	0.3
Tridecane	1.17	Benzeneacetic acid, .alpha.-oxo-, ethyl ester	0.54
Cycloundecane, 1,1,2-trimethyl-	0.42	Benzeneacetic acid, ethyl ester	1.02
Tridecane, 2-methyl-	1.06	Benzenepropanoic acid, ethyl ester	0.8
Pentadecane	2.48	Decanoic acid, ethyl ester	0.76
Hexadecane	2.18	Methyl tetradecanoate	0.54
Cyclopropane, nonyl-	1.33	Pentadecanoic acid, 14-methyl-, methyl ester	0.56
Tetracosane	0.49	Octadecanoic acid, ethyl ester	12.86
Tridecane, 1-iodo-	0.68	Pentadecanoic acid, ethyl ester	1.71
Docosane	0.31	Hexadecanoic acid, methyl ester	0.77
Alkenes	**3.45**	Ethyl 9-hexadecenoate	2.52
1-Dodecene	0.59	E-11-Hexadecenoic acid, ethyl ester	1.02
9-Octadecene, (E)-	0.6	Hexadecanoic acid, ethyl ester	11.85
1-Tridecene	1.21	Heptadecanoic acid, ethyl ester	0.84
7-Hexadecene, (Z)-	0.47	Octadecanoic acid, methyl ester	0.49
9-Eicosene, (E)-	0.58	(E)-9-Octadecenoic acid ethyl ester	4.1
Aromatics	**9.38**	9-Octadecenoic acid, ethyl ester	1.89
Toluene	0.15	Sulfurous acid, 2-ethylhexyl hexyl ester	0.68
Ethylbenzene	1.16	Docosanoic acid, ethyl ester	1.28
Benzene, 1,3-dimethyl-	5.56	**Nitrogenated compounds**	**14.11**
Benzene, methoxy-	0.26	Pyridine, 2-methyl-	0.24
Benzene, (1-methylethyl)-	0.63	Pentanenitrile, 4-methyl-	0.23
Benzene, propyl-	0.26	Pyridine, 3-methyl-	0.42
Benzene, ethoxy-	0.18	Pyridine, 2,6-dimethyl-	0.11
Benzene, butyl-	0.33	Pyridine, 2,4-dimethyl-	0.22
Benzene, pentyl-	0.46	Pyridine, 3-ethyl-	0.16
Benzene, hexyl-	0.39	Benzonitrile	0.27

Compounds	% Relative Content	Compounds	% Relative Content
Ketones	**2.52**	Pyridine, 2,4,6-trimethyl-	1.33
2,5-Pyrrolidinedione, 1-methyl-	0.98	1H-Pyrrole, 2-ethyl-4-methyl-	0.51
2,5-Pyrrolidinedione, 1-ethyl-	0.53	1H-Pyrrole, 2,3,5-trimethyl-	0.24
2(1H)-Pyridinone, 1-ethyl-	0.15	2-Ethyl-3,5-dimethylpyridine	0.5
1H-Isoindole-1,3(2H)-dione, 2-methyl-	0.86	1H-Pyrrole, 3-ethyl-2,4-dimethyl-	1.28
Phenols	**6.95**	1H-Pyrrole, 3,4-diethyl-2-methyl-	0.93
Phenol	1.19	1H-Indole, 3-methyl-	1.33
Phenol, 2-methyl-	0.46	Benzonitrile, 2,4,6-trimethyl-	1.33
Phenol, 3-methyl-	2.48	1H-Indole, 2,3-dimethyl-	0.65
Phenol, 2,3-dimethyl-	0.81	Pentadecanenitrile	0.38
Phenol, 4-ethyl-	0.82	Hexadecanenitrile	1.17
Phenol, 2-ethyl-6-methyl-	1.19	Heptadecanenitrile	0.64
Ester	**48.59**	N-Methyldodecanamide	0.91
Propanoic acid, 2-methyl-, ethyl ester	0.24	Silanediamine, 1,1-dimethyl-N,N'-diphenyl-	0.1
Butanoic acid, ethyl ester	0.19	N,N-Dimethyldodecanamide	1.16

The proposed mechanism for the conversion of alkanes to ester (Fig. 5f) is coined from the principle of oxidative conversion of hydrocarbons. In this work, the CO_2 and the CO from decarboxylation and decarbonylation processes were considered oxidants. The total alkane fractions, including those derived from the reduction of alkenes by hydrogenation (Fig. 5c), was transformed to carboxylic acids and eventually to ester compounds with Ni/HZSM-5 catalyst. Alkanes to carboxylic acids transformation passed through series of reactions with CO, water, and nitrile compounds. The CO was produced in situ or via CO_2 reduction, while water and nitrile compounds were components of the feed bio-oil. Though this pathway is strange in many bio-oil upgrading studies, its uniqueness can be attributed to differences of feed bio-oil components particularly acid fractions. The propose mechanism is supported by conversion of alkanes to carboxylic acids in aqueous medium (Kirillova et al. 2009) whereby the mixture of water/acetonitrile solvent and alkanes with the presence of CO resulted to carboxylic acids conversion.

Figure 5: Proposed mechanisms of the products in the upgrading of sewage-derived bio-oil using Ni/HZSM-5 catalyst with ethanol co-feed and hydrogen gas



Water and nitrile compounds are essentially important because carboxylation practically does not proceed when one is absent. The work of Kirillova et al. further indicated that incorporating metal catalyst as promoter in the reaction resulted to higher yields (up to 72%) of carboxylic acids. Apart from the work of Kirillova et al. where $S_2O_8^{2-}$ is added in the reaction system, the study of using CO alone (without any O_2 source) in the carboxylation of methane with vanadate metal catalyst at 100°C indicated formation of carboxylic acid at an increasing trend with increased in CO pressure (Nizova et al. 1998). It is explained that CO_2 can serve as carboxylating reagent by first reducing it to CO. This means that even without any

oxidizing agent like $S_2O_8^{2-}$, carboxylic production would likely proceed at higher yield especially if metal is added in the system. On the other hand, the study of oxidative conversion of alkanes, alkenes and alcohols with carbon dioxide over Mn oxide-based catalyst (Krylov, Mamedov, and Mirzabekova 1995) proposed that oxygen generated during CO_2 reduction can possibly serve as oxidant in the partial oxidation and dehydration. All of these claims from previous works strengthened the likelihood of these unprecedented proposed mechanisms.

The use of HZSM-5 catalyst was also investigated in small alkanes with the presence of CO and H_2O for the formation of carboxylic acids which indicated high conversion at 300°C temperature (Luzgin et al. 2000). In their work, the proposed mechanisms of propane and isobutene carboxylation proceed with the presence of H_2, CO and H_2O. However, this mechanism less likely occurred in this current work considering that neither carboxylic acid nor ester was observed in the control run with Ni/HZSM-5 and H_2.

Conclusions

Production of bio-oil from digested sewage sludge and the subsequent catalytic upgrading come up with the following conclusions:

1. Digested sewage sludge from the domestic wastewater treatment plant is a good biomass for fast pyrolysis owing to high recovery of bio-oil with high calorific value.
2. Heating value of the fast pyrolysis bio-oil of 36.43 MJ/kg is higher compared to many bio-oil derived from 2nd generation (e.g. agriwastes) bio-fuels. The subsequent upgraded bio-oil has heating value above the required biodiesel standard as stipulated in ASTM D6751.
3. Contrary to most bio-oil, the pH of bio-oil derived from sludge is generally neutral to alkaline. Catalytic upgrading process makes the bio-oil alkaline, a pH beneficial for engine application without risk of corrosion in vessels and pipelines.
4. Desirable high fractions of alkanes and alkenes were found in the fast pyrolysis bio-oil. Small fractions of aromatics and alcohols are likewise detected. Catalytic upgrading resulted to conversion of bio-oil components into desirable esters.
5. Bio-oil from fast pyrolysis contains nitrogenated and oxygenated compounds which require upgrading. Catalytic upgrading using Ni/HZSM-5 catalyst reduced the feed bio-oil nitrogen fractions most likely due to ammonia formation when nitrile compounds were hydrolyzed which eventually proceed to hydrodenitrogenation. Oxygenated compounds were reduced due the complex reactions that took place including decarbonylation, hydrogenation and decarboxylation.
6. The use of Ni/HZSM-5 catalyst with ethanol co-feed in catalytic upgrading generally improved the properties of the bio-oil through complex processes such as hydrogenation, decarbonylation, decarboxylation, cracking, hydrocracking, and hydrodenitrogenation and hydrodeoxygenation.
7. Because the raw bio-oil has no acid; the presence of water, nitrile compounds and carbon oxides (CO and CO_2 produced in situ) were likely responsible for the production of ester compounds found in the upgraded bio-oil.
8. Fast pyrolysis and catalytic upgrading studies demonstrated that waste digested sewage sludge is a valuable energy resource as evident in the production of a better quality bio-oil generally ready for transportation/ energetic applications.

Acknowledgement

The author is very grateful to Texas A&M University (TAMU), USA through the Bio-Energy Testing and Analysis (BETA) Laboratory and the Department of Science and Technology (DOST), Philippines through the Technology through the Engineering Research and Development for Technology (ERDT) for financial support. Special thanks to MOSCAT administration for granting a study leave to the author.

References

Adjaye, J.D., and N.N. Bakhshi. 1995. “Production of Hydrocarbons by Catalytic Upgrading of a Fast Pyrolysis Bio-Oil. Part I: Conversion over Various Catalysts.” Fuel Processing Technology 45 (December): 161–183. doi:10.1016/0378-3820(95)00034-5. http://linkinghub.elsevier.com/retrieve/pii/0378382095000345.

Bezerra, Marcos Almeida, Ricardo Erthal Santelli, Eliane Padua Oliveira, Leonardo Silveira Villar, and Luciane Amélia Escaleira. 2008. “Response Surface Methodology (RSM) as a Tool for Optimization in Analytical Chemistry.” Talanta 76 (September 15): 965–977. doi:10.1016/j.talanta.2008.05.019. http://www.ncbi.nlm.nih.gov/pubmed/18761143.

Bhaskar, Thallada, Balagurumurthy Bhavya, Rawel Singh, Desavath Viswanath Naik, Ajay Kumar, and Hari Bhagwan Goyal. 2011. “Thermochemical Conversion of Biomass to Biofuels.” In Biofuels: Alternative Feedstocks and Conversion Processes, 51–77. India: Elsevier Inc. doi:10.1016/B978-0-12-385099-7.00003-6.

Bulushev, Dmitri A., and Julian R.H. Ross. 2011. “Catalysis for Conversion of Biomass to Fuels via Pyrolysis and Gasification: A Review.” Catalysis Today 171 (August): 1–13. doi:10.1016/j.cattod.2011.02.005. http://linkinghub.elsevier.com/retrieve/pii/S092058611100099X.

Butler, Eoin, Ger Devlin, Dietrich Meier, and Kevin McDonnell. 2011. “A Review of Recent Laboratory Research and Commercial Developments in Fast Pyrolysis and Upgrading.” Renewable and Sustainable Energy Reviews 15 (8) (October): 4171–4186. doi:10.1016/j.rser.2011.07.035. http://linkinghub.elsevier.com/retrieve/pii/S1364032111002759.

Buys, Bastian R, A Klapwijk, H Elissen, and W H Rulkens. 2008. “Development of a Test Method to Assess the Sludge Reduction Potential of Aquatic Organisms in Activated Sludge.” Bioresource Technology 99 (November): 8360–8366. doi:10.1016/j.biortech.2008.02.041. http://www.ncbi.nlm.nih.gov/pubmed/18407493.

Cao, Jing-Pei, Liu-Yun Li, Kayoko Morishita, Xian-Bin Xiao, Xiao-Yan Zhao, Xian-Yong Wei, and Takayuki Takarada. 2013. “Nitrogen Transformations during Fast Pyrolysis of Sewage Sludge.” Fuel 104 (February): 1–6. doi:10.1016/j.fuel.2010.08.015. http://linkinghub.elsevier.com/retrieve/pii/S001623611000431X.

Cao, Qing, Ke-Chang Xie, Wei-Ren Bao, and Shu-Guang Shen. 2004. “Pyrolytic Behavior of Waste Corn Cob.” Bioresource Technology 94 (August): 83–89. doi:10.1016/j.biortech.2003.10.031. http://www.ncbi.nlm.nih.gov/pubmed/15081491.

Capareda, Sergio C. 2013. Introduction to Biomass Energy Conversions. 1st editio. Texas, United States of America: CRC Press, Taylor and Francis Group.

Capunitan, Jewel a., and Sergio C. Capareda. 2012. “Assessing the Potential for Biofuel Production of Corn Stover Pyrolysis Using a Pressurized Batch Reactor.” Fuel 95 (May): 563–572. doi:10.1016/j.fuel.2011.12.029. http://linkinghub.elsevier.com/retrieve/pii/S0016236111007988.

Coates, John. 2000. “Interpretation of Infrared Spectra, A Practical Approach.” In Encyclopedia of Analytical Chemistry, edited by R.A. Meyers, 10815–10837. Newtown, USA: John Wiley & Sons Ltd.

Domínguez, A., J.A. Menéndez, M. Inguanzo, and J.J. Pis. 2005. “Investigations into the Characteristics of Oils Produced from Microwave Pyrolysis of Sewage Sludge.” Fuel Processing Technology 86 (May): 1007–1020. doi:10.1016/j.fuproc.2004.11.009.

http://linkinghub.elsevier.com/retrieve/pii/S0378382004002073.
Dutta, Ratna, Ujjaini Sarkar, and Alakananda Mukherjee. 2014. “Extraction of Oil from Crotalaria Juncea Seeds in a Modified Soxhlet Apparatus: Physical and Chemical Characterization of a Prospective Bio-Fuel.” Fuel 116 (January): 794–802. doi:10.1016/j.fuel.2013.08.056.
http://linkinghub.elsevier.com/retrieve/pii/S0016236113007849.
Fisk, Courtney A., Tonya Morgan, Yaying Ji, Mark Crocker, Czarena Crofcheck, and Sam A. Lewis. 2009. “Bio-Oil Upgrading over Platinum Catalysts Using in Situ Generated Hydrogen.” Applied Catalysis A: General 358 (2) (May 1): 150–156. doi:10.1016/j.apcata.2009.02.006.
http://linkinghub.elsevier.com/retrieve/pii/S0926860X09001161.
Fonts, Isabel, Gloria Gea, Manuel Azuara, Javier Ábrego, and Jesús Arauzo. 2012. “Sewage Sludge Pyrolysis for Liquid Production: A Review.” Renewable and Sustainable Energy Reviews 16 (5) (June): 2781–2805. doi:10.1016/j.rser.2012.02.070.
http://linkinghub.elsevier.com/retrieve/pii/S1364032112001657.
Francioso, Ornella, Maria Teresa Rodriguez-Estrada, Daniela Montecchio, Cesare Salomoni, Armando Caputo, and Domenico Palenzona. 2010. “Chemical Characterization of Municipal Wastewater Sludges Produced by Two-Phase Anaerobic Digestion for Biogas Production.” Journal of Hazardous Materials 175 (March 15): 740–746. doi:10.1016/j.jhazmat.2009.10.071.
http://www.ncbi.nlm.nih.gov/pubmed/19931274.
Fytili, D., and A. Zabaniotou. 2008. “Utilization of Sewage Sludge in EU Application of Old and New methods—A Review.” Renewable and Sustainable Energy Reviews 12 (January): 116–140. doi:10.1016/j.rser.2006.05.014.
http://linkinghub.elsevier.com/retrieve/pii/S1364032106000827.
Gao, Ningbo, Juanjuan Li, Benyu Qi, Aimin Li, Yue Duan, and Ze Wang. 2014. “Thermal Analysis and Products Distribution of Dried Sewage Sludge Pyrolysis.” Journal of Analytical and Applied Pyrolysis 105 (January): 43–48. doi:10.1016/j.jaap.2013.10.002.
http://linkinghub.elsevier.com/retrieve/pii/S0165237013002155.
Gasco, G., M.J. Cueto, and A. Méndez. 2007. “The Effect of Acid Treatment on the Pyrolysis Behavior of Sewage Sludges.” Journal of Analytical and Applied Pyrolysis 80 (October): 496–501. doi:10.1016/j.jaap.2007.03.009.
http://linkinghub.elsevier.com/retrieve/pii/S0165237007000514.
Gil-Lalaguna, Noemı, Isabel Fonts, Gloria Gea, Marıa B Murillo, and Lazaro Luisa. 2010. “Reduction of Water Content in Sewage Sludge Pyrolysis Liquid by Selective Online Condensation of the Vapors.” Energy & Fuels 24: 6555–6564. doi:10.1021/ef101024j.
Guo, Xiujuan, Shurong Wang, Zuogang Guo, Qian Liu, Zhongyang Luo, and Kefa Cen. 2010. “Pyrolysis Characteristics of Bio-Oil Fractions Separated by Molecular Distillation.” Applied Energy 87 (September): 2892–2898. doi:10.1016/j.apenergy.2009.10.004.
http://linkinghub.elsevier.com/retrieve/pii/S0306261909004322.
Houillon, G., and O. Jolliet. 2005. “Life Cycle Assessment of Processes for the Treatment of Wastewater Urban Sludge: Energy and Global Warming Analysis.” Journal of Cleaner Production 13 (February): 287–299. doi:10.1016/j.jclepro.2004.02.022.
http://linkinghub.elsevier.com/retrieve/pii/S0959652604000976.
Inguanzo, M, A Domınguez, J A Menendez, C G Blanco, and J J Pis. 2002. “On the Pyrolysis of Sewage Sludge: The Influence of Pyrolysis Conditions on Solid, Liquid and Gas Fractions.” Journal of Analytical and Applied Pyrolysis 63: 209–222.
Izhar, Shamsul, Satoshi Uehara, Noboru Yoshida, Yugo Yamamoto, Tohru Morioka, and Masatoshi Nagai. 2012. “Hydrodenitrogenation of Fast Pyrolysis Bio-Oil Derived from Sewage Sludge on NiMo/Al2O3 Sulfide Catalyst.” Fuel Processing Technology 101 (September): 10–15. doi:10.1016/j.fuproc.2012.03.012.
http://linkinghub.elsevier.com/retrieve/pii/S0378382012001051.
Jacobson, Kathlene, Kalpana C. Maheria, Ajay Kumar Dalai, and Ajay Kumar Dalai. 2013. “Bio-Oil Valorization: A Review.” Renewable and Sustainable Energy Reviews 23 (July): 91–106. doi:10.1016/j.rser.2013.02.036. http://linkinghub.elsevier.com/retrieve/pii/S136403211300138X.
Kelessidis, Alexandros, and Athanasios S Stasinakis. 2012. “Comparative Study of the Methods

Used for Treatment and Final Disposal of Sewage Sludge in European Countries." Waste Management 32 (6) (June): 1186–1195. doi:10.1016/j.wasman.2012.01.012. http://www.ncbi.nlm.nih.gov/pubmed/22336390.
Kirillova, Marina V, Alexander M Kirillov, Maxim L Kuznetsov, José A. L. Silva, João J R Fraústo da Silva, and Armando J L Pombeiro. 2009. "Alkanes to Carboxylic Acids in Aqueous Medium: Metal-Free and Metal-Promoted Highly Efficient and Mild Conversions." Chemical Communications (May 7): 2353–2355. doi:10.1039/b900853e. http://www.ncbi.nlm.nih.gov/pubmed/19377683.
Krylov, O.V., A. Kh. Mamedov, and S. R. Mirzabekova. 1995. "Catalytic Oxidation of Hydrocarbons and Alcohols by Carbon Dioxide on Oxide Catalysts." Industrial and Engineering Chemistry Research 34: 474–482.
Laturnus, Frank, Karin von Arnold, and Christian Gron. 2007. "Organic Contaminants from Sewage Sludge Applied to Agricultural Soils: False Alarm Regarding Possible Problems for Food Safety?" Environmental Science and Pollution Research International 14: 53–60.
Liu, Jing-yong, and Shui-yu Sun. 2013. "Total Concentrations and Different Fractions of Heavy Metals in Sewage Sludge from Guangzhou, China." Transactions of Nonferrous Metals Society of China 23 (8) (August): 2397–2407. doi:10.1016/S1003-6326(13)62747-8. http://linkinghub.elsevier.com/retrieve/pii/S1003632613627478.
Luo, Zhongyang, Shurong Wang, and Kefa Cen. 2005. "A Model of Wood Flash Pyrolysis in Fluidized Bed Reactor." Renewable Energy 30 (March): 377–392. doi:10.1016/j.renene.2004.03.019. http://linkinghub.elsevier.com/retrieve/pii/S0960148104001259.
Luzgin, Mv, Alexander G. Stepanov, Alain Sassi, and Jean Sommer. 2000. "Formation of Carboxylic Acids from Small Alkanes in Zeolite H-ZSM-5." Chemistry - A European Journal 6 (13) (July 3): 2368–2376. http://www.ncbi.nlm.nih.gov/pubmed/10939739.
Mahmood, Talat, and Allan Elliott. 2006. "A Review of Secondary Sludge Reduction Technologies for the Pulp and Paper Industry." Water Research 40 (June): 2093–2112. doi:10.1016/j.watres.2006.04.001. http://www.ncbi.nlm.nih.gov/pubmed/16701789.
Manara, P, and A Zabaniotou. 2012. "Towards Sewage Sludge Based Biofuels via Thermochemical Conversion – A Review." Renewable and Sustainable Energy Reviews 16 (5): 2566–2582. doi:10.1016/j.rser.2012.01.074. http://dx.doi.org/10.1016/j.rser.2012.01.074.
Massoth, F E, P Politzer, M C Concha, J S Murray, J Jakowski, and Jack Simons. 2006. "Catalytic Hydrodeoxygenation of Methyl-Substituted Phenols: Correlations of Kinetic Parameters with Molecular Properties." Journal of Physical Chemistry B 110 (July 27): 14283–14291. doi:10.1021/jp057332g. http://www.ncbi.nlm.nih.gov/pubmed/16854134.
Mortensen, P.M. M, J.-D. Grunwaldt, P.A. A Jensen, K.G. G Knudsen, and A.D. D Jensen. 2011. "A Review of Catalytic Upgrading of Bio-Oil to Engine Fuels." Applied Catalysis A: General 407 (1-2) (November): 1–19. doi:10.1016/j.apcata.2011.08.046. http://linkinghub.elsevier.com/retrieve/pii/S0926860X11005138.
Nizova, Galina V., Suss-Fink Georg, Sandrine Stanislas, and Georgiy B. Shul'pin. 1998. "Carboxylation of Methane with CO or CO2 in Aqueous Solution Catalysed by Vanadium Complexes." Chemical Communication Journal: 1885–1886.
NREL. 2009. "Biodiesel Handling and Use Guide." Tennessee, USA. http://www.osti.gov/bridge.
Park, Hyun Ju, Hyeon Su Heo, Young-Kwon Park, Jin-Heong Yim, Jong-Ki Jeon, Junhong Park, Changkook Ryu, et al. 2010. "Clean Bio-Oil Production from Fast Pyrolysis of Sewage Sludge: Effects of Reaction Conditions and Metal Oxide Catalysts." Bioresource Technology 101 (January): S83–S85. doi:10.1016/j.biortech.2009.06.103. http://dx.doi.org/10.1016/j.biortech.2009.12.078.
Perla, Donna, William Brandes, Herb Fredrickson, Fran Kremer, Andy Miller, Susan Thornloe, David Kirchgessner, et al. 2006. "Recovering the Value of Waste for Environmental and Energy Sustainability." USA. http://www.epa.gov/etopetop/pubs/reportRecoveringValueWaste092506.pdf.
Pokoo-Aikins, Grace, Aubrey Heath, Ray a. Mentzer, M. Sam Mannan, William J. Rogers, and

Mahmoud M. El-Halwagi. 2010. “A Multi-Criteria Approach to Screening Alternatives for Converting Sewage Sludge to Biodiesel.” Journal of Loss Prevention in the Process Industries 23 (3) (May): 412–420. doi:10.1016/j.jlp.2010.01.005. http://linkinghub.elsevier.com/retrieve/pii/S0950423010000173.
Pokorna, E., N. Postelmans, P. Jenicek, S. Schreurs, R. Carleer, and J. Yperman. 2009. “Study of Bio-Oils and Solids from Flash Pyrolysis of Sewage Sludges.” Fuel 88 (August): 1344–1350. doi:10.1016/j.fuel.2009.02.020. http://linkinghub.elsevier.com/retrieve/pii/S0016236109000891.
Research & Education Association. 2007. The Organic Chemistry Problem Solver. New Jersey, USA: Research & Education Association, Inc.
Santos, Bjorn S. 2013. “Liquid -Phase Processing of Fast Pyrolysis Bio-Oil Using Pt/HZSM-5 Catalyst.” Texas A&M University.
Singh, Kunwar P, Shikha Gupta, Arun K Singh, and Sarita Sinha. 2011. “Optimizing Adsorption of Crystal Violet Dye from Water by Magnetic Nanocomposite Using Response Surface Modeling Approach.” Journal of Hazardous Materials 186: 1462–1473. doi:10.1016/j.jhazmat.2010.12.032. http://dx.doi.org/10.1016/j.jhazmat.2010.12.032.
Suranani, Srinath, and Venkat Reddy Goli. 2011. “Combustion Characteristics of Sawdust in a Bubbling Fluidized Bed.” In 2011 International Conference on Chemistry and Chemical Process, 10:167–172. Singapore: IACSIT Press.
Walter, I, F Martínez, and V Cala. 2006. “Heavy Metal Speciation and Phytotoxic Effects of Three Representative Sewage Sludges for Agricultural Uses.” Environmental Pollution 139 (February): 507–514. doi:10.1016/j.envpol.2005.05.020. http://www.ncbi.nlm.nih.gov/pubmed/16112313.
Werther, J., and T. Ogada. 1999. “Sewage Sludge Combustion.” Progress in Energy and Combustion Science 25 (February): 55–116. doi:10.1016/S0360-1285(98)00020-3. http://linkinghub.elsevier.com/retrieve/pii/S0360128598000203.
Westerhof, Roel J. M., D. Wim F. Brilman, Manuel Garcia-Perez, Zhouhong Wang, Stijn R. G. Oudenhoven, Wim P. M. Van Swaaij, and Sascha R. A. Kersten. 2011. “Fractional Condensation of Biomass Pyrolysis Vapors.” Energy & Fuels 25: 1817–1829. http://cat.inist.fr/?aModele=afficheN&cpsidt=24129621.
Xiu, Shuangning, and Abolghasem Shahbazi. 2012. “Bio-Oil Production and Upgrading Research: A Review.” Renewable and Sustainable Energy Reviews 16 (7) (September): 4406–4414. doi:10.1016/j.rser.2012.04.028. http://linkinghub.elsevier.com/retrieve/pii/S1364032112003036.
Yin, Chungen. 2012. “Microwave-Assisted Pyrolysis of Biomass for Liquid Biofuels Production.” Bioresource Technology 120 (September): 273–284. doi:10.1016/j.biortech.2012.06.016. http://www.ncbi.nlm.nih.gov/pubmed/22771019.
Yuan, Xingzhong, Huajun Huang, Guangming Zeng, Hui Li, Jingyu Wang, Chunfei Zhou, Huina Zhu, Xiaokai Pei, Zhantao Zhifeng Liu, and Zhantao Zhifeng Liu. 2011. “Total Concentrations and Chemical Speciation of Heavy Metals in Liquefaction Residues of Sewage Sludge.” Bioresource Technology 102 (5) (March): 4104–10. doi:10.1016/j.biortech.2010.12.055. http://www.ncbi.nlm.nih.gov/pubmed/21211964.
Yunquan, Yang, Lou He’an, Tong Gangsheng, Kevin J. Smith, and Tye Ching Thian. 2008. “Hydrodeoxygenation of Phenolic Model Compounds over MoS2 Catalysts with Different Structures.” Chinese Journal of Chemical Engineering 16 (5): 733–739.
Zhang, Le, Ronghou Liu, Renzhan Yin, and Yuanfei Mei. 2013. “Upgrading of Bio-Oil from Biomass Fast Pyrolysis in China: A Review.” Renewable and Sustainable Energy Reviews 24 (August): 66–72. doi:10.1016/j.rser.2013.03.027. http://linkinghub.elsevier.com/retrieve/pii/S136403211300186X.
Zhang, Xinghua, Tiejun Wang, Longlong Ma, Qi Zhang, and Ting Jiang. 2013. “Hydrotreatment of Bio-Oil over Ni-Based Catalyst.” Bioresource Technology 127: 306–311.

Antibacterial Activity and Mechanisms of Action of Essential Oil Extracted from *Litsea Cubeba* in North Vietnam Toward *Escherichia Coli* ATCC 25922

NGUYEN HAI VAN[1,2], MEILE JEAN-CHRISTOPHE[2], LEBRUN MARC[2], SON CHU-KY[1], SARTER SAMIRA[2]

[1] Hanoi University of Science and Technology, Vietnam
[2] CIRAD, France

Paper abstract

Overuse of antibiotics in aquaculture has been associated with bacterial resistance which has been increasingly reported, not only in pathogenic bacteria, but also in commensal and environmental bacteria, acting as a reservoir of resistant determinants. In this context, a growing interest has arisen towards herbal therapy for reducing the use of antibiotics, enhancing fish resistance and improving growth and feed efficiency in order to strengthen the sustainability of aquaculture. May Chang (*Litsea cubeba*) is a small tree (5-8 meters) belonging to the Lauraceae family and it is widely distributed in Southern China, Japan, Taiwan and South East Asia region. The ethnobotanical use of *L. cubeba* is widespread in several countries of South East Asia and in China but its antimicrobial activity has been underexplored so far. The aims of this study were to characterize the main compounds, the antibacterial activity as well as the mechanism of action *L. cubeba* essential oil (EO) from leaves and fruits harvested in Phu Tho province (North Vietnam). Indeed, fresh leaves/fruits were submitted to hydrodistillation for 4h using a Clevenger-type apparatus in order to obtain EO. The chemical constituents of EOs of *L. cubeba* were analyzed by gas chromatography couple with mass spectroscopy (GC-MS).

Our results showed that EOs obtained were a complex mixture of numerous compounds with the dominant of linalool (95 %) in leaves oil and citral (40%) in fruits oil. The inhibitory effects of both EOs were tested against *Escherichia coli* ATCC 25922 by using microbroth dilution assay in 96-well microplates. Leaves oil showed higher antimicrobial activity than fruits oil (Minimum Inhibitory Concentration was 0.96 mg mL^{-1} when compared with 5.25 mg mL^{-1}) which might be dependent on the EO composition. The EOs have a variety of targets, particularly the membrane and cytoplasm, and in certain situations, they completely alter the morphology of the cells. In this research, the mechanisms of action of *L. cubeba* EOs were explored on *E. coli* model using fluorescence microscopy to observe the membrane integrity, metabolism of DNA, cell morphology and cell viability. *L. cubeba* EOs induced damage to the cell membrane structures, lost and replication of DNA. Cells treated with *L. cubeba* EOs for 2h showed a strong inhibition of cell division and appeared as filamentous cells when compared to the control with a decreased by 60 % of *E. coli* viability. Therefore, our results could be of great potential for the discovery of plant candidates for sustainable therapy in aquaculture including fish and shrimp to improve food quality and safety.

Keywords: *Litsea cubeba*, essential oil, antimicrobial activity, mechanism of action, sustainable therapy, aquaculture

Pandan Leaves Extract (*Pandanous Amaryllifolius Roxb.*) as Natural Antioxidant in Selected Vegetable Oils During Accelerated Storage

ANDRIATI NINGRUM

Department of Food Science and Agricultural Product Technology, Faculty of Agricultural Technology, Gadjah Mada University, Indonesia

Abstract

The application of pandan leaves extract (PLE) were observed as a natural antioxidant in red palm oil (RPO) and soybean oil (SO) using accelerated oxidation at 70ºC for 3 days. DPPH (1,1-Diphenyl-2-picrylhydrazyl) scavenging activities, β-carotene linolenic assay, rancimat analysis were performed to analyze the effect of PLE and time incubation (0 day and 3 days storage) to the antioxidant activities in RPO and SO. During the accelerated storage, antioxidant activities of PLE in RPO and SO are commonly significantly difference ($p < 0.05$). The PLE extract (optimum concentration is 0.18%) gave the best result to retard oil oxidation in RPO and SO. These results gave a promising application of pandan leaves extract as a good natural alternative to be applied in several vegetable oils.

Keywords: Antioxidant, Pandan Leaves, Soybean oil, Red Palm Oil
Abbreviation: PLE : Pandan leave extract, RPO: Red Palm Oil; SO: Soybean Oil

Introduction

Several antioxidants are important ingredients in food processing since they have the ability to protect foods, containing oils and fats from deteoriation or damage caused by free radicals and reactive oxygen species (Casarotti and Jorge 2012). Peroxidation of lipid causes not only deterioration of flavor and taste of oil containing foods, but also several chronic diseases (Carocho and Ferreira 2013; Mohd Esa et al. 2013). Several synthetic antioxidants are widely used in the food industry; however, their utilization has been questioned because of safety aspect (Casarotti and Jorge 2012). Several research were investigated to fulfill the need of new natural antioxidants to use as safe ingredients in the food industry. So that, nowadays there is a growing interest in the use of natural antioxidants to reduce or replace the synthetic antioxidants.

Several species are used in cooking, medicine purpose with high antioxidant activity (Casarotti and Jorge 2012; Maisuthisakul, Pongsawatmanit, and Gordon 2006; Jorge, N., Ré 2013). Addition of several plants extract to prevent oxidation were applied in several lipid model system e.g. addition of grape marc extract to the hazelnut paste, addition of pandan leaves to refined bleached deodorized palm oil during deep fat frying, addition of oregano and thyme in soybean oil, rosemary in soybean oil, etc (Spigno et al. 2013; Jorge, N., Ré 2013; Gámez-Meza et al. 2009). From the safety aspects, these several plants contains several antioxidants which will give positive effects for human health also. One of the example is rosemary extract has been proved in Europe where the European commission published Directive 2010/67/EU and informed in the safety of rosemary extracts when it is used as antioxidant in foods (Vicente et al. 2012).

Pandan leaves have been used for deep frying process also in several Asia cuisine. The genus name Pandanus is derived from the Indonesian name of the tree,

pandan. In several Asia countries, pandan leaves, names given include pandan wangi (Malaysian), daun pandan (Indonesian), bai toey or toey hom (Thai), taey (Khmer), tey ban, tey hom (Laotian), dua thom (Vietnamese), and ban yan le (Chinese)(Wongpornchai 2004). The distribution of pandan leaves is found over Southern India, the Southeast Asia peninsular, Indonesia and Western New Guinea (Wongpornchai 2004). The plants grow in clumps and have thin and sharp leaves at the edge where the form is like sword, fragrant odor

Pandan leaves, commonly known as pandan, are often used to give a refreshing, fragrant flavour to both sweet and savoury South-East-Asian dishes. These leaves are also traditionally used for traditional medicine. As a traditional herbal this leaves are generally used for traditional medicine especially to heal the typhus illness in Indonesia (Roosita et al. 2008). The leaves have been investigated to have antimicrobial activities on the preservation of stored milk (Khusniati and Widyastuti 2008). One biomolecule in pandan leaves were isolated from fresh pandan leaves which is unglycosylate protein, lectin, called pandanin. Several alkaloids such as pandanmine, pandamerilactones with pyrroline derived structures are also found in the leaves with is potential activity for health (Ooi, Sun, and Ooi 2004). Pandanin is a single polypeptide chain and molecular weight of 8 KDa and exhibits hemagglutinating activity toward rabbit erythrocytes. Pandanin also have a potential antiviral activities against human viruses e.g. herpes simplex virus type=1 (HSV-1) and influenza virus (H1N1) (Ooi, Sun, and Ooi 2004).

There are real interest in the use of natural antioxidant to improve the oxidative stability of food lipids. The assessment of antioxidant capacity and effectiveness is a relevant task in food chemistry, for the choice of the best antioxidant to be added to a certain oxidisable food product and also for the prediction of its shelf life (Mancebo-Campos, Salvador, and Fregapane 2014). Several natural antioxidative substances from edible herbs have been applied in several vegetable oils to enhance the antioxidant activity (Nor et al. 2008; Maisuthisakul, Pongsawatmanit, and Gordon 2006; Andreani et al. 2013; Gámez-Meza et al. 2009; Vieira, Ré, and Jorge 2011; Vicente et al. 2012; Rodrigues et al. 2012; Casarotti and Jorge 2012; Merrill et al. 2008). These natural antioxidant are believed to be safer and also potential for health benefits. Like other green leafy vegetables, pandan leaves are also known as potential source of several lipophilic antioxidant e.g. β-carotene, vitamin E, phenolic compounds, ascorbic acid (Isabelle et al. 2010). Besides the lipophilic antioxidants, it also contain quercetin, alkaloids, fatty acids and ester (Nor et al. 2008). Several phenolic compounds and flavonoids compounds also found in pandan leaves (Ghasemzadeh and Jaafar 2013b).

Objective

Our objective is to investigate the influence of addition of pandan leaves to the quality of palm oil and soybean oil during accelerated oxidation at 70ºC for 3 days. Quality aspects mean the antioxidant capacity e.g. DPPH radical scavenging activity, β-carotene bleaching activity and rancimat analysis.

Materials and Methods

Materials

Fresh pandan leaves, red palm oil and soybean oil were purchased in local market. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and linolenic acid were purchased from Sigma Aldrich. A UV-VIS Spectrophotometer Hitachi U 2900 with 1 cm matched cells was used for all absorbance measurements. Rancimat analysis by Metrohm model 892, Herisan, Switzerland

Preparation of herbs extract

The preparation of herb extract were performed by drying of pandan leaves in a hot air ovet at 45°C for 1 day. Afterwards, 6 g of dried leaves were extracted with 300 mL ethanol overnight at room temperature. The solvent were removed by rotary evaporator.

Accelerated Storage

The palm oil and soybean oil were heated to 65ºC before addition of pandan leave extract (at 0% (Control), 0.06%, 0.12% and 0.18%) and were stirred to ensure that it completely dissolved. The quality property and oxidative stability of the oil were tested during the accelerated storage conditions by modified oven test at a fixed temperature of 70ºC for 3 days. 1 day of storage represents 1 month of storage at ambient temperature (Razmkhah et al. 2013). Duplicate sample will be stored in glass bottles, respectively with both similarity wrapped in alumunium foil and stored incubator at 70ºC for 3 days (Razmkhah et al. 2013).

DPPH radical scavenging assay

The free radical scavenging activity was assayed based on the reduction of DPPH radicals in methanol, which causes an absorbance drop at 517 nm. The extracts activity against the DPPH radical will be evaluated according to previously described method with slight modification ("Antioxidative Properties of Pandanus Amaryllifolius Leaf Extracts in Accelerated Oxidation and Deep Frying Studies"). 4 mL DPPH in methanol (0.1 mM) was added 1 mL of sample solution (Control, 0.06%, 0.12%, 0.18% v/v). After 20 min, the absorbance will be measured at 517 nm. Radical scavenging activity will be expressed as the percentage of inhibition.

β-carotene linolenic assay

The antioxidant activity is measured by the ability of a compound to minimize the coupled oxidation of linolenic acid and β-carotene in an emulsified aqueous system. The β-carotene bleaching (BCB) assay will be performed according to previously described method with slight modification (Razmkhah et al. 2013). First, 3 mL of β-carotene (50 mg/50 mL in chloroform) was added to linolenic acid (40 mg) and Tween 40 (400 mg). The chloroform was evaporated, followed by addition of deionized water (100 mL) to prepare the β-carotene linolenic mixture. The resulting emulsion will be added to a tube containing 100µL sample. Absorbance will be immediately measured at 470 nm against a blank (first result) and then all the test tubes will be incubated at 70ºC for 20 min. The oxidation of the emulsion will be monitored spectrophotometrically at 470 nm after 20 min (second result). AOA will be expressed as the inhibition percentage with reference to the control after incubation.

%AOA = 100 (DR control – DR sample) / DR control
%AOA : Antioxidant Activity
DR control : Degradation Rate control
DR sample : Degradation Rate sample

Rancimat Analysis

The Rancimat analysis (Metrohm model 892, Herisan, Switzerland) will determine the induction periods by measuring the increase in the volatile acidic by products released from the oxidizing oil at 120°C with an air flow rate 20 L/h. The concentration of the degradation products, which are transferred into distilled water, is assessed by measuring the conductivity. OSI (Oxidative Stability Index) was defined as the time (h) of maximum change of the rate of oxidation. The longer induction periods suggest stronger activity for the added pandan leaves extract in the oil. The relative activity will be expressed by the protection factor (PF), which is calculated by dividing the induction period of the oil with added antioxidants by that of the control (the oil sample alone)

Statistical Analysis

Each experiment was repeated in duplicate, and the data were then analyzed by SPSS software program. The general linear model procedure was applied and Duncan's multiple range test was used to compare mean values at $p< 0.05$. Mean values and pooled standard error of the mean

Results and Discussion

Firstly, antioxidant activity of pandan leaves extracts were investigated by the free radical scavenging (DPPH method). It was evaluated using DPPH methods which consist in the capacity of several concentrations of PLE in RPO to scavenge the DPPH free radicals. If the quantity of compounds with antioxidant properties are higher so the discoloration of the violet solution of DPPH are higher also which is indicative of higher antioxidant potential (Rodrigues et al. 2012). Based on the free radical scavenging activities of PLE in RPO during the storage are described in Table 1. In Table 1, it is possible to observe that the pandan leaves concentration gave significant result of scavenging activities in RPO ($p<0.05$). The optimum value of scavenging activity achieved with 0.18% of PLE at the beginning of measurement (0 day). The scavenging activity of PLE extracts is probably related to the concentration of antioxidants in pandan leaves (Ghasemzadeh and Jaafar 2013a). In pandan leaves several antioxidants are present e.g. flavonoids, phenolics compounds will affect the result of scavenging activity of PLE in RPO.

Table 1: Scavenging activity of PLE in RPO

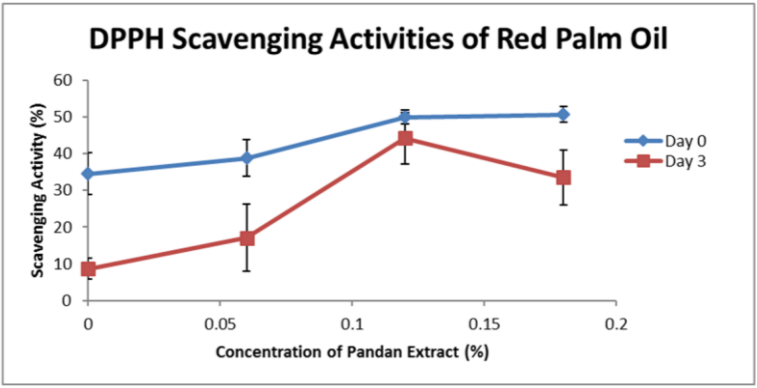
	Scavenging Activity (%)	
PLE (%)	Day 0	Day 3
0	34.524	8.686
0.06	38.782	17.096
0.12	49.954	44.210
0.18	50.641	33.502

PLE: Pandan Leaves Extract; RPO: Red Palm Oil

The different level of PLE and time storage gave a significant results to the scavenging activities ($p<0.05$). Based on the Figure 1, the scavenging activities of several concentration of PLE in RPO will be decrease during the storage. Significant higher losses in antioxidant properties were observed in all samples during the storage ($p<0.05$). The samples with addition of PLE displayed higher capacity to counteract or prevent the oxidation. The DPPH value of PLE in RPO showed as significant decrease on days 0, 3 of storage that can be explained by the positive correlation between the depletion of several antioxidants inside PLE in RPO and loss of antioxidant activity of the oil. From this results indicate that the

antioxidants tested were unable to remain stable after the storage in accelerated condition at 70ºC for 3 days.

Figure 1: Scavenging Activities of PLE in RPO. Data points represent mean± standard error (n=2).



We investigated also the scavenging activities of PLE in SO (Table 2). Similar with the PLE in RPO, the optimum concentration of PLE in SO gave the best result for DPPH scavenging activity in the concentration of 0.18% at 0 day and after 3 days storage. This results however showed that the DPPH value of PLE in SO had not significantly decrease on day 0 to day 3 in control and 0.06%. This could imply that several antioxidants compounds other than phenolic and flavonoids were probably involved in inhibiting the DPPH radicals (Razmkhah et al. 2013). Similarity in the previous investigation, sometime no significant increase in the scavenging activity of addition of antioxidant in the oil than control. It has been proven that even though antioxidants will generally show moderate to high redox activity, not all redox-active compounds generated during heating of sample could be antioxidants as they might not be capable of transferring electrons to radicals (Razmkhah et al. 2013).

Table 2: Scavenging Activity of PLE in SO

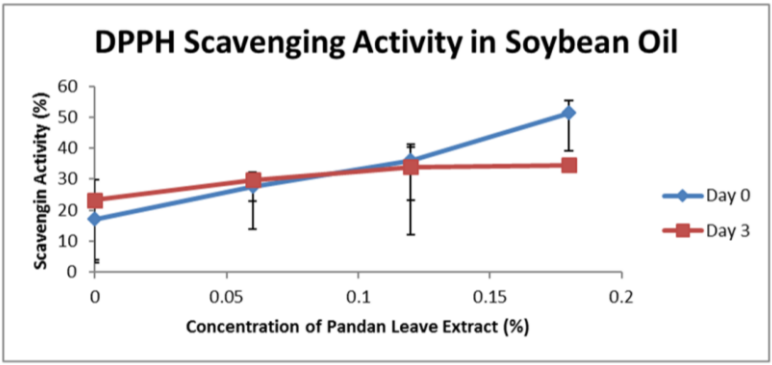
	Scavenging Activity (%)	
PLE (%)	**Day 0**	**Day 3**
0	17.092	23.194
0.06	27.626	29.781
0.12	36.035	33.910
0.18	51.396	34.578

PLE: Pandan Leaves Extratct; SO: Soybean Oil

Figure 2 shows the scavenging activities of several concentration of PLE in SO. If we compare with the scavenging activities of PLE in RPO (Figure 1), during the accelerated oxidation at 70º C for 3 days the scavenging activities will decrease significantly ($p<0.05$). The carotenoids in RPO gave a significant results to the DPPH scavenging activity after accelerated oxidation at 70º C for 3 days. Carotenoids are substance that easy to oxidize because several pro oxidants e.g. air, light and high temperature, etc (Britton, Liaaen-Jensen, and Pfander 2004). During the storage the carotenoids in RPO are easy to oxidize. This will cause the reduction of DPPH scavenging activity in RPO rather than in SO.

It has been proven that the antioxidant activity of PLE in RPO and SO is mainly contributed by the concentration of several antioxidant compounds e.g. phenolic compounds and flavonoid compounds in pandan leaves (Ghasemzadeh and Jaafar 2013b). The concentration of these content will decrease during storage as a result of thermal and oxidative distress, the DPPH radical scavenging activity will commonly decrease also. According to the results, the initial scavenging activity of PLE in RPO and SO are higher than the control (PLE 0%) suggested that the natural antioxidant compounds within PLE were capable of scavenging free radicals.

Figure 2: Scavenging Activities of PLE in SO. Data points represent mean± standard error (n=2).



The antioxidant activity was also determined by the linoleic acid model system. The results are described in Table 3 for RPO. In Table 3, it is possible to observe that during the storage the antioxidant activities in different level of PLE concentration in RPO were decreasing and significantly different ($p<0.05$). Based on Table 3, the optimum concentration of PLE extract in this analysis is 0.18%. The higher concentration of PLE extract in RPO will increase the antioxidant activity in the linolenic acid model.

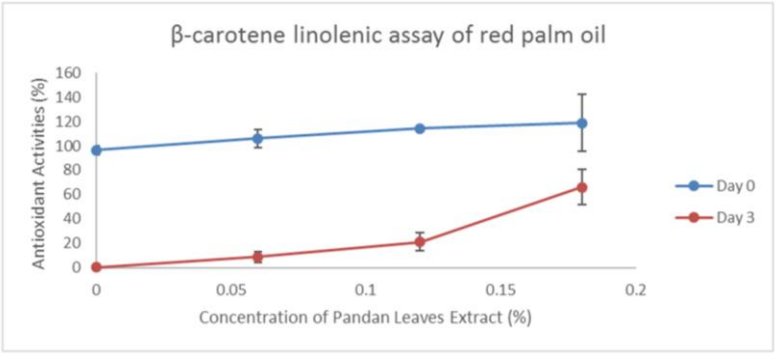
Table 3: Antioxidant activity by the linolenic acid model of PLE in RPO

	AOA (%)	
PLE (%)	**Day 0**	**Day 3**
0	96.319	0.179
0.06	106.041	8.776
0.12	114.475	21.021
0.18	118.703	66.155

PLE: Pandan Leaves Extratct; RPO: Red Palm Oil AOA: Antioxidant Activity

Based on Figure 3, we can observe that the optimum concentration of 0.18 % of PLE showed optimum antioxidant activity in RPO. With this linoleic acid model, the samples were analyzed for 20 minutes, and the spectrophotometric measurements were performed. From this data, it was possible to describe the degradation kinetic curve of β-carotene and to evaluate the effectiveness of the antioxidant added in RPO and SO. The presence of several antioxidants in the system protect the linoleic acid extending the period of formation of free radical.

Figure 3: Antioxidant activity by the linolenic acid model of PLE in RPO. Data points represent mean± standard error (n=2).



The antioxidant activity by the linoleic acid model system of PLE in SO is described in Table 4. Similar pattern with the antioxidant activity of PLE in RPO, it is possible to observe that during the storage the antioxidant activities in different level of PLE concentration in SO will decrease and significantly different ($p<0.05$). Similar with athe antioxidant activity of PLE in RPO, the optimum concentration of PLE in SO is 0.16% (Table 4).

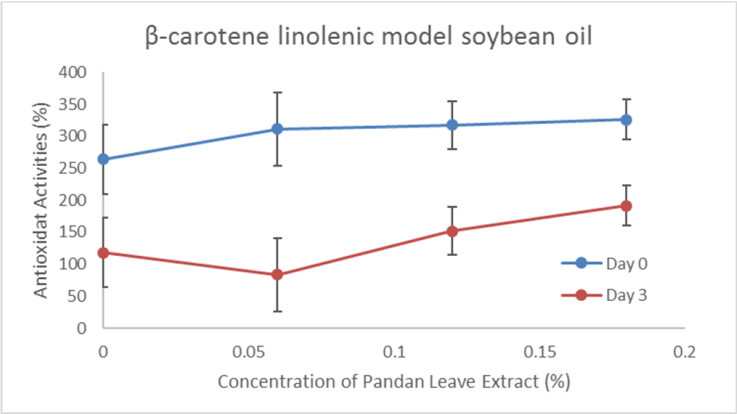
Table 4. Antioxidant activity by the linolenic acid model of PLE in SO

	AOA (%)	
PLE (%)	Day 0	Day 3
0	264.364	117.935
0.06	311.403	83.880
0.12	317.225	151.936
0.18	325.797	191.875

PLE: Pandan Leaves Extratct; SO: Soybean Oil AOA: Antioxidant Activity

Based on Figure 4, we can observe that the optimum antioxidant activity of PLE in SO is 0.16%. The presence of several antioxidants of PLE in the system protect the linoleic acid extending the period of formation of free radical.

Figure 4: Antioxidant activity by the linolenic acid model of PLE in SO. Data points represent mean± standard error (n=2).



Application of PLE in RPO was analyzed by rancimat method also. The global oxidative stability is an important parameter in the evaluation of oils resistance to oxidation under heating (Rodrigues et al. 2012). Its evaluation by means of forced oxidation measurements under heating as in the Rancimat analysis, is a way to compare samples. The rancimat analysis was performed at 120ºC and the induction period or called as oxidative stability index (OSI) was evaluated until the end point of stability for oil samples. The rancimat method has been performed to measure the oxidative stability index of synthetic and natural antioxidants. This method will measure the increases in electric conductivity that arises when fats and oil are oxidized to shorter fatty acids under accelerated conditions of heat and aeration (Gámez-Meza et al. 2009).

Oxidative Stability Index (OSI) is closely dependent on the degree of unsaturation of the oil and also on the presence of antioxidants, which are likewise affected by the temperature (Casarotti and Jorge 2012). This analysis measures the total volatile carbonyl compounds, which are secondary products from lipid oxidation. This method also evaluates the oil's resistance to oxidation (Gámez-Meza et al. 2009). Oxidation was slow until reach a point where the oxidation accelerated and became very rapid. The length of the time before rapid acceleration of oxidation is the measure of the resistance to oxidation and is commonly referred to as the induction period or oxidative stability index.

Based on Table 5, there was a significant reduction of OSI during the storage of the oil with or without antioxidants ($p<0.05$). From this results indicate that the antioxidants tested were unable to remain stable after the storage in accelerated condition at 70ºC for 3 days.

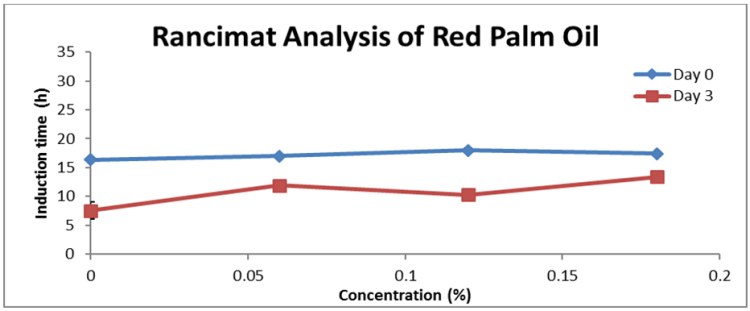
Table 5: Rancimat analysis of PLE in RPO

PLE (%)	Induction time (h)	
	Day 0	Day 3
0	16.340	7.505
0.06	16.965	11.915
0.12	18.005	10.255
0.18	17.440	13.415

PLE: Pandan Leaves Extract; RPO: Red Palm Oil

Application of PLE in |RPO were analyzed with rancimat analysis. In Figure 5, the induction time of several concentration of PLE in RPO is not significantly different ($p<0.05$). The accelerated storage of RPO in 3 days caused the decrease of induction time in several concentration of PLE in RPO.

Figure 5: Induction time of PLE in RPO. Data points represent mean± standard error (n=2).



We also investigated the rancimat analysis of PLE in SO. Almost similar pattern with PLE in RPO, in several concentration of PLE in SO showed not significantly results. The 3 days storage at 70ºC of several concentration of PLE in SO gave a significant results to the induction time ($p<0.05$).

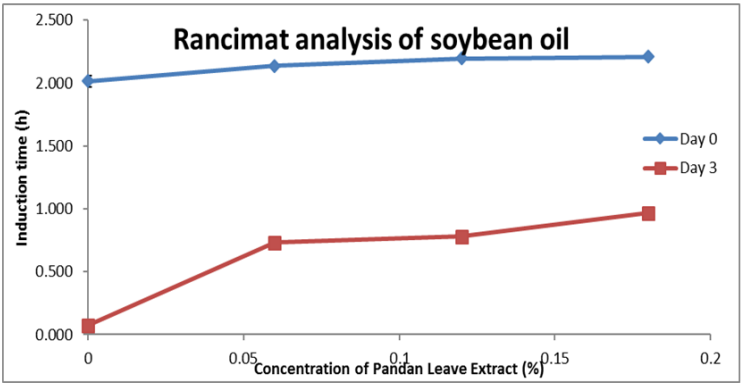
Table 6: Rancimat analysis of PLE in SO

	Induction time (h)	
PLE (%)	**Day 0**	**Day 3**
0	2.015	0.075
0.06	2.135	0.730
0.12	2.195	0.780
0.18	2.210	0.965

PLE: Pandan Leaves Extratct; SO: Soybean Oil

Figure 6 shows the rancimat analysis of several concentration of PLE is SO. Rancimat analysis showed that the induction time during the time storage decreased the induction time. This results also gave a same pattern same like in RPO.

Figure 6: Induction time of PLE in SO. Data points represent mean± standard error (n=2).



These observation by Rancimat analysis are commonly in accordance with all the chemical assays analyzed and discussed previously. The addition of the PLE in RPO and SO allowed better resistance to the oxidative process once that the PLE possesses several antioxidant substance (Ghasemzadeh and Jaafar 2013b).
Protection factor of several concentration of pandan leaves in RPO is investigated during the storage (0 day and 3 days storage). The protective effect of pandan leaves will increase after the time incubation for 3 days.

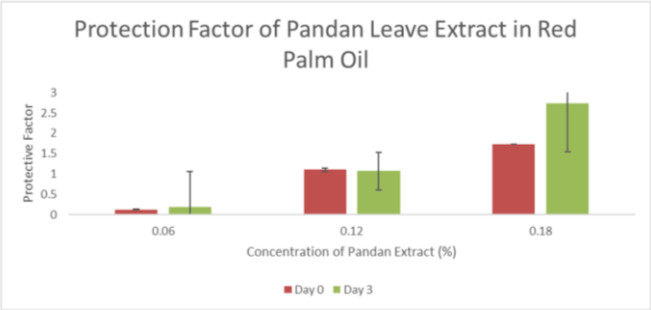
Table 7. Protection factor of PLE in RPO

	Induction time (h)	
PLE (%)	Day 0	Day 3
0.06	1.038	2.284
0.12	1.102	1.734
0.18	1.067	2.739

PLE: Pandan Leaves Extratct; RPO: Red Palm Oil

Figure 7 shows the protection factor of pandan leaves. The increase of different concentration of pandan extract gave mostly a higher protective effect. And this protective effect also will increase during the accelerated oxidation at 70ºC for 3 days. It is interesting to note that the protection factor for pandan leaves are significantly higher than the control ($p<0.05$). Several herbs extract e.g. rosemary extract can give a better protection in several vegetable oil in Rancimat test (Vicente et al. 2012).

Figure 7: Different of protection factor of PLE in RPO. Data points represent mean± standard error (n=2).



The protection factor of several concentration of pandan leaves in SO is also investigated during the storage (0 day and 3 days storage). Same like in RPO, the protective effect of pandan leaves in SO will increase after the time incubation at 70ºC for 3 days.

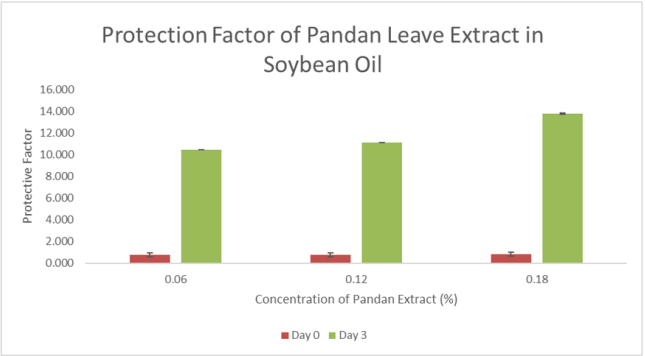
Table 8. Protection factor of PLE in SO

	Induction time (h)	
PLE (%)	**Day 0**	**Day 3**
0.06	0.771	10.429
0.12	0.793	11.143
0.18	0.811	13.786

PLE: Pandan Leaves Extract; SO: Soybean Oil

Figure 8 shows the protection factor of pandan leaves in SO. The increase of different concentration of pandan extract gave mostly a higher protective effect. And this protective effect also will increase during the accelerated oxidation at 70ºC for 3 days. The protection factor was observed in SO rather the RPO because the more unsaturated fatty acids content, a higher antioxidant activity was displayed and with that higher oxidative stability even at higher exposition times during incubation.

Figure 8: Protection factor of PLE in SO. Data points represent mean± standard error (n=2).



Conclusion

Our results for antioxidant activity were in agreement with previously published results which conclude that pandan leaves can increase the antioxidant activity as indicated in various in vitro assays, for instance DPPH radical-scavenging activity, the linolenic model an rancimat analysis in RPO and SO. In conclusion, the results obtained in this present work found that the PLE is a good source of natural compounds that can be used as stabilizers in RPO and SO towards oxidation. Application of pandan leaves has been investigated also in refined bleached and deodorized palm oil (RBD palm oil) where the carotenoids have been bleached and removed in the oil (Nor et al. 2008). The synergistic aspect of pandan leaves in the refined and bleached palm oil were enhanced by the interaction between several antioxidant compounds in pandan leaves and existing tocopherols and tocotrienolds in RBD palm oil (Nor et al. 2008). Pandan leaves are very easy to cultivate and with several positive antioxidant compounds. Due to this reasons, it is promising to add pandan leaves extract as natural antioxidant in several vegetable oils.

Acknowledgment

The author wish to thank to Dr. Matthias Scheriner for his kindly supervision, OeAD for technology grant scholarship and Institute of Food Science for supporting this project.

Reference

Andreani, Stéphane, Marie-Cecile De Cian, Julien Paolini, Jean-Marie Desjobert, Jean Costa, and Alain Muselli. 2013. “Chemical Variability and Antioxidant Activity of Limbarda Crithmoides L. Essential Oil from Corsica.” Chemistry & Biodiversity 10 (11) (November): 2061–77. doi:10.1002/cbdv.201300109. http://www.ncbi.nlm.nih.gov/pubmed/24243615.

“Antioxidative Properties of Pandanus Amaryllifolius Leaf Extracts in Accelerated Oxidation and Deep Frying Studies.” http://ac.els-cdn.com/S0308814608001672/1-s2.0-S0308814608001672-main.pdf?_tid=059088f4-d745-11e2-99a8-00000aab0f6b&acdnat=1371470402_ede1d09105fad48c9e5e5c24d4d1c452.

Britton, G, S Liaaen-Jensen, and H. Pfander. 2004. Carotenoids. Edited by George Britton. 4th ed. Switzerland: Birkhauser.

Carocho, Márcio, and Isabel C F R Ferreira. 2013. “A Review on Antioxidants, Prooxidants and Related Controversy: Natural and Synthetic Compounds, Screening and Analysis Methodologies and Future Perspectives.” Food and Chemical Toxicology : An International Journal Published for the British Industrial Biological Research Association 51 (January): 15–25. doi:10.1016/j.fct.2012.09.021. http://www.ncbi.nlm.nih.gov/pubmed/23017782.

Casarotti, Sabrina Neves, and Neuza Jorge. 2012. “Antioxidant Activity of Rosemary Extract in Soybean Oil Under Thermoxidation.” Journal of Food Processing and Preservation (July 19): no–no. doi:10.1111/j.1745-4549.2012.00755.x. http://doi.wiley.com/10.1111/j.1745-4549.2012.00755.x.

Gámez-Meza, Nohemi, Juan Antonio Noriega-Rodríguez, Lilia Leyva-Carrillo, Jesus Ortega-García, Lorena Bringas-Alvarado, Hugo Sergio García, and Luis Angel Medina-Juárez. 2009. “Antioxidant Activity Comparison of Thompson Grape Pomace Extract, Rosemary and Tocopherols in Soybean Oil.” Journal of Food Processing and Preservation 33 (2009) (August): 110–120. doi:10.1111/j.1745-4549.2008.00285.x. http://doi.wiley.com/10.1111/j.1745-4549.2008.00285.x.

Ghasemzadeh, Ali, and Hawa Ze Jaafar. 2013a. “Profiling of Phenolic Compounds and Their Antioxidant and Anticancer Activities in Pandan (Pandanus Amaryllifolius Roxb.) Extracts from Different Locations of Malaysia.” BMC Complementary and Alternative Medicine 13 (January): 341. doi:10.1186/1472-6882-13-341. http://www.ncbi.nlm.nih.gov/pubmed/24289290.

———. 2013b. “Profiling of Phenolic Compounds and Their Antioxidant and Anticancer Activity in Pandan (Pandanus Amaryllifolius Roxb.) Extracts from Different Locations of Malaysia.” BMC Complementary and Alternative Medicine 13 (1) (December 1): 341. doi:10.1186/1472-6882-13-341. http://www.ncbi.nlm.nih.gov/pubmed/24289290.

Isabelle, Mia, Bee Lan Lee, Meng Thiam Lim, Woon-Puay Koh, Dejian Huang, and Choon Nam Ong. 2010. “Antioxidant Activity and Profiles of Common Vegetables in Singapore.” Food Chemistry 120 (4) (June): 993–1003. doi:10.1016/j.foodchem.2009.11.038. http://linkinghub.elsevier.com/retrieve/pii/S030881460901348X.

Jorge, N., Ré, P.V.D. 2013. “Protective Effect of Oregano (Origanum Vulgar L.) and Thyme (Thymus Vulgaris L.) Oleoresins in Stored Soybean Oil.” Nutrition and Food Science 43 (3): 189–195.

Khusniati, Tatik, and Yantyati Widyastuti. 2008. “The Preservation of Milk with The Addition of Antibacterial and Aromatic Supplements Produced in Indonesia.” Biotropia 15 (1): 50–64.

Maisuthisakul, Pitchaon, Rungnaphar Pongsawatmanit, and Michael H Gordon. 2006. “Antioxidant Properties of Teaw (Cratoxylum Formosum Dyer) Extract in Soybean Oil and Emulsions.” Journal of Agricultural and Food Chemistry 54 (7) (April 5): 2719–25. doi:10.1021/jf052396+. http://www.ncbi.nlm.nih.gov/pubmed/16569066.

Mancebo-Campos, Vanessa, María Desamparados Salvador, and Giuseppe Fregapane. 2014. “Antioxidant Capacity of Individual and Combined Virgin Olive Oil Minor Compounds Evaluated at Mild Temperature (25 and 40°C) as Compared to Accelerated and Antiradical Assays.” Food Chemistry 150 (May): 374–381. doi:10.1016/j.foodchem.2013.10.162. http://linkinghub.elsevier.com/retrieve/pii/S0308814613016221.

Merrill, Lilon I., Oscar a. Pike, Lynn V. Ogden, and Michael L. Dunn. 2008. “Oxidative Stability of Conventional and High-Oleic Vegetable Oils with Added Antioxidants.” Journal of the American Oil Chemists’ Society 85 (8) (June 3): 771–776. doi:10.1007/s11746-008-1256-4. http://link.springer.com/10.1007/s11746-008-1256-4.

Mohd Esa, Norhaizan, Khairul-Kamilah Abdul Kadir, Zulkhairi Amom, and Azrina Azlan. 2013. “Antioxidant Activity of White Rice, Brown Rice and Germinated Brown Rice (in Vivo and in Vitro) and the Effects on Lipid Peroxidation and Liver Enzymes in Hyperlipidaemic Rabbits.” Food Chemistry 141 (2) (November 15): 1306–12. doi:10.1016/j.foodchem.2013.03.086. http://www.ncbi.nlm.nih.gov/pubmed/23790918.

Nor, Fatihanim Mohd, Suhaila Mohamed, Nor Aini Idris, and Razali Ismail. 2008. “Antioxidative Properties of Pandanus Amaryllifolius Leaf Extracts in Accelerated Oxidation and Deep Frying Studies.” Food Chemistry 110 (2) (September): 319–327. doi:10.1016/j.foodchem.2008.02.004. http://www.sciencedirect.com/science/article/pii/S0308814608001672.

Ooi, Linda S M, Samuel S M Sun, and Vincent E C Ooi. 2004. “Purification and Characterization of a New Antiviral Protein from the Leaves of Pandanus Amaryllifolius (Pandanaceae).” The International Journal of Biochemistry & Cell Biology 36 (8) (August): 1440–6. doi:10.1016/j.biocel.2004.01.015. http://www.ncbi.nlm.nih.gov/pubmed/15147723.

Razmkhah, Sara, Chin-Ping Tan, Kamariah Long, and Kar-Lin Nyam. 2013. “Quality Changes and Antioxidant Properties of Microencapsulated Kenaf (Hibiscus Cannabinus L.) Seed Oil During Accelerated Storage.” Journal of the American Oil Chemists’ Society 90 (12) (August 28): 1859–1867. doi:10.1007/s11746-013-2333-x. http://link.springer.com/10.1007/s11746-013-2333-x.

Rodrigues, Nuno, Ricardo Malheiro, Susana Casal, M Carmen Asensio-S-Manzanera, Albino Bento, and José Alberto Pereira. 2012. “Influence of Spike Lavender (Lavandula Latifolia Med.) Essential Oil in the Quality, Stability and Composition of Soybean Oil during Microwave Heating.” Food and Chemical Toxicology : An International Journal Published for the British Industrial Biological Research Association 50 (8) (August): 2894–901. doi:10.1016/j.fct.2012.05.020. http://www.ncbi.nlm.nih.gov/pubmed/22659463.

Roosita, Katrin, Clara M. Kusharto, Makiko Sekiyama, Yulian Fachrurozi, and Ryutaro Ohtsuka. 2008. “Medicinal Plants Used by the Villagers of a Sundanese Community in West Java, Indonesia.” Journal of Ethnopharmacology 115 (1) (January): 72–81.

doi:10.1016/j.jep.2007.09.010.
http://www.sciencedirect.com/science/article/pii/S0378874107004825.
Spigno, Giorgia, Francesco Donsì, Danila Amendola, Mariarenata Sessa, Giovanna Ferrari, and D. Marco De Faveri. 2013. “Nanoencapsulation Systems to Improve Solubility and Antioxidant Efficiency of a Grape Marc Extract into Hazelnut Paste.” Journal of Food Engineering 114 (2) (January): 207–214. doi:10.1016/j.jfoodeng.2012.08.014.
http://linkinghub.elsevier.com/retrieve/pii/S0260877412003974.
Vicente, Gonzalo, Diana Martín, Mónica R García-Risco, Tiziana Fornari, and Guillermo Reglero. 2012. “Supercritical Carbon Dioxide Extraction of Antioxidants from Rosemary (Rosmarinus Officinalis) Leaves for Use in Edible Vegetable Oils.” Journal of Oleo Science 61 (12) (January): 689–97. http://www.ncbi.nlm.nih.gov/pubmed/23196869.
Vieira, Patrícia, D E L Ré, and Neuza Jorge. 2011. “Antioxidant Potential of Oregano (Oreganum Vulgare L .), Basil (Ocimum Basilicum L .) and Thyme (Thymus Vulgaris L .): Application of Oleoresins in Vegetable Oil” 31 (4): 955–959.
Wongpornchai, S. 2004. Pandan Wangi. Edited by K V Peter and Abington Hall. Cambridge, UK: Woodhead Publishing in Food Science.

Effect of Current Post-harvest Management Practices and Identification of Fungal Species in Peanut Kernels in Myanmar

SU HLAING CHEIN, AVISHEK DATTA, ANIL KUMAR ANAL

Asian Institute of Technology, Thailand

Abstract

Fungal contamination is a major challenge in peanuts (*Arachis hypogaea* L.) production and trade besides posing health challenges to humans and animals. *Aspergillus* spp. produce highly toxic and carcinogenic chemical substances known as aflatoxins which are the major toxins affecting the quality of peanuts meant for human consumption. In Myanmar, poor handling and inadequate storage conditions, prevalent in many villages and collectors, along with poor processing can detrimentally affect crop contents. The majority of farmers, traders and consumers in the study area are not currently aware of the fungal contamination in peanut kernels. Fungal contamination can occur in the field during post harvest handling, drying and storage. The aim of this study was to identify the fungus species and to determine the effect of postharvest management and the factors associated fungus contamination in peanuts from one of the peanut production region in the state of Magway region, Myanmar. Peanut kernels were sampled at farmer stores, buyer collector'sstores, and the wholesaler' stores. A total of 85 isolates were morphologically identified as *Aspergillus niger*, *Aspergillus terreus*, *Aspergillus* spp. and *Penicillium ctirinum*. Isolates were further confirmed by DNA sequencing analyzing using species-specific primers. It has been found that 22 isolates belonged to *A. niger*, *A. terreus* (10), *Aspergillus* spp. (43) and another (10) isolates were *Penicillium citrinum*. Analysis of peanut kernel samples showed that *Aspergillus* spp. was the most frequent fungus species (40 %) followed by *A. niger* (14.7%), *A. terreus* (13.69 %), and *P. citrinum* (5.24 %) respectively at the farmer stores. These results indicate a potential risk of fungal contamination if good storage practices are not applied and also the farmers did not have a good understanding of the problems and of the importance of managing of fungal contamination

Keywords: Peanut, Postharvest handling, *Aspergillus*fungi, *Penicillium* fungi, Molecular, DNA, Sequencing

Introduction

In Myanmar, peanuts are used in the fabrication of sweets, candies and pastes and mainly as a raw material in oil production.Contamination of peanuts with aflatoxins in the field is difficult to control because of the influence of climatic conditions, mainly relative humidity and temperature. In addition, factors such as soil moisture content, damage caused by insects, mineral deficiency, and stress play an important role in fungal contamination. One important factor that contributes to the contamination of stored peanuts is the high moisture content of peanut grains during postharvest drying and the inability to maintain adequate moisture during storage (Davison, Whitaker, and Dickens 1982).According to (Ezekiel 2008), poor storage condition, storage period, temperature, humidity levels and suitable climate could lead to infection caused by various storage fungi, such as Aspergillus species, Penicillium species, Curvularia species, Alternarla alternate, Cladosporium

cladosporiodes and Phoma species (Reddy 2009).The problem of fungal growth and aflatoxin contamination of foodstuffs remains especially in developing countries where handling and storage technologies are still being developed. Moisture content is the most important factor affecting fungal growth in stored peanuts.
Post-harvest deterioration in groundnut (*Arachis hypogaea* L.) is largely due to mould development, especially by the *Aspergillus* section *Flavi* group of fungi. Aflatoxins produced by *Aspergillus flavus, Aspergillus parasiticus*, and others in this group are secondary metabolites with carcinogenic, estrogenic, teratogenic, and immunosuppressive effects (Klich 2009). Contamination of groundnuts by these fungi occurs at both pre- and post-harvest stages, leading to aflatoxin contamination. Post-harvest stages generally include cleaning, grading, transportation, storage, processing, packaging, and retailing at the market (Kimatu 2012). Some of the factors affecting aflatoxin contamination in food grains are harvesting, drying, and storage methods as well as moisture content, insect damage, and physical damage (Kaaya and Warren 2005). Polypropylene bags are now being used, but because these are not airtight, groundnut pods are still susceptible to fungal and aflatoxin contamination (Hell and Udoh 2000). A major precaution in bag storage is to ensure that bags are clean when reusing them, especially when used for maize, rice, sorghum, beans, or cocoa. This is because reused bags often contain *A. flavus* spores (Awuah and Kpodo 1996). Grain moisture content, mould growth, aflatoxins, and free fatty acid content were significantly higher in pods stored in jute bags than in those stored in polyethylene-doubled jute bags (Bulaong and Dharmaputra 2002). Moisture and temperature are the main factors that influence post-harvest contamination of stored commodities by *A. flavus* (Hell and Mutegi 2011). Sorting of kernels to remove discoloured or damaged/shrivelled pods is often recommended to minimise aflatoxin levels (Afolabi2006, Awuah and Kpodo 1996 and Park 2002). Accurate identification of fungal pathogen is in many cases, a pre-requisite for effective management of the diseases they cause and for ecological or population genetics studies (Gherbawy and Voigt 2010). However, these fungal species are much more similar to each other and accurate identification to species level could not be possible. Hence, it is paramount that their morphological and molecular characteristics with respect to DNA presences are investigated, using the methods of fungal isolation and screening making use of macro- and microscopic analysis, fungal DNA extraction, polymerase chain reaction (PCR) and an agarose gel electrophoresis.
The objective of this study was to find the factors, which are the effect of post harvest management of peanut kernels in Magway district, and to identify the different *Aspergillus* and *Penicillium* species employing with the help of Molecular method, from peanut kernels. The sequencing of ITS gene and Beta-tubulin gene was efficient in differing *Aspergillus* spp.which can be distinguished by Morphological method.

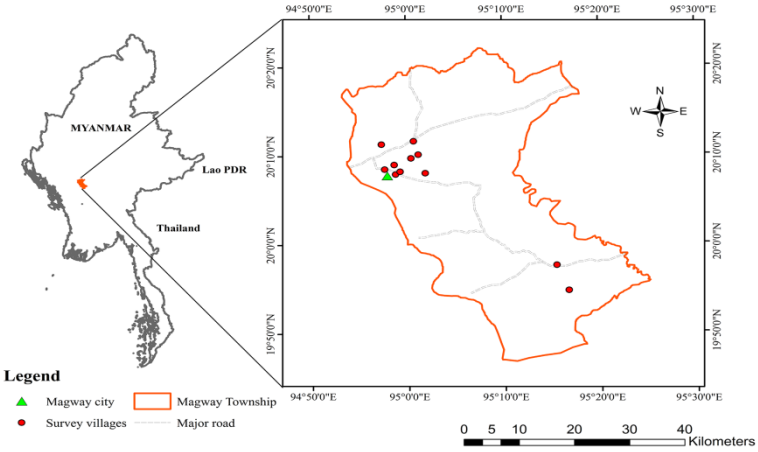
Materials and Methods

Study place, survey questionnaires and peanuts sampling

The study area was located in one of the peanut production region in the state of Magway, Myanmar (Fig 1). The survey questionnaires which are postharvest handling and storage practices were conducted with one hundred and seventy eight respondents included (140 farmers, 28 collectors and 10 small wholesalers) and interviewed with one hundred and forty farmers from elevenvillages and twenty eight respondents from collectors and ten respondents from small wholesalers at Magway district. The peanut kernels which were stored for 3 days dried, 4 days dried, 7 days dried and 10 daysdried at farm were collected in the field and farmer's

house during the natural drying moisture content of less than 12 percent and stored peanut kernels of moisture content of less than 9 from farmer's stored houses, collectors and wholesalers. A total samples of 75 places(35sites from farmersand 20placesfrom collectors and20 places wholesaler's stores), each weighing 300g were collected.

Figure 1: The study area with the survey sites



Determination of moisture content

The moisture content of peanut kernel samples was determined using WILE 55 Grain moisture meter (Finland) in the field and storage house during data collection.

Isolation of fungi from peanut samples

Fungi were isolated and identified in accordance with good laboratory practice. Direct inoculation was used for fungal isolation from kernels. A 50 g aliquot of each sample was disinfected with 0.4 % sodium hypochloride solution for 3 min, followed by three washes with sterilie distilled water in order to eliminate external contaminants. After disinfection, the kernels were inoculated into Petri dishes containing Dichloran 18 agar medium. Three plates containing 8kernels were prepared for each sample, corresponding to a total of 24 kernels per sample. The plates were incubated at 37°C for 5 days and the results are expressed as total percentage of kernels infected with fungi.

Molecular identification of the isolated fungi

DNA extraction and PCR amplification

Genomic DNA was extracted from fresh mycelia using E.Z.N.A Forensic DNA isolation Kit (Omega Bio-Tek), according to the manufacturer's instruction.The beta-tubulin gene and the internal transcribed spacer (ITS) region were amplified in a 50 μl reaction volume containing 1X buffer, 2.5 mM MgCl2, 0.2 mM dNTPs, 0.2 μM of each primer (Bt2a and Bt2b) and (ITS 5 and ITS 4), and 1U Taq DNA polymerase. The PCR temperature profile began with an initial denaturation at 94°C for 3 min,

followed by 35 cycles of 94°C for 1 min, 53°C for 1 min and 72°C for 2 min for beta-tubulin gene and with an initial denaturation at 96°C for 2 min, followed by 35 cycles of 96°C for 1 min, 53°C for 1 min and 72°C for 1.5 min for internal transcribed gene .The final extension was carried out for 10 min at 72°C for each.

Gel Electrophoresis and Sequencing

PCR product was checked by 1% agarose gel electrophoresis stained with ethidium bromide, and visualized under ultraviolet (UV) trans illuminator. The PCR product was sent to sequenced for both directions on an automated DNA sequencer (Macrogen inc., KoreaBLAST result (Beta- Tubulin gene) of Bt2a / Bt2b primers pair and (Internal Transcribe Spacer (ITS) of ITS5/ITS4 primers pair.

Statistical analysis

Pearson's correlation coefficient and the respective (p) valueswere evaluated by using the SAS 9.0 software in order to evaluate the association between the growth of different fungi species in kernels and each storehouse. Pearson's correlation coefficient instead of Spearman's correlation coefficient was used since many samples were contaminated with fungi.

Results and Discussion

Factors Associated with fungus contamination of peanuts in the study area, Magway District

All farmers from the study area planted local varieties of peanuts, with most of seeds sources from farmers. Farmers harvested their peanuts at 60 to 120 days after planting depending on the varieties. During planting, all the respondents used fertilizer, such as urea and TSP. Weed control was conducted by manually.Based on their experiences, farmers could determine when pods are fully dried for storage and packed in polypropylene bags. Cleaning dried peanut pods, sorting out damaged, immature pods are very important practices before storage. All farmers from that study area stored under farm condition without pre cleaning damaged and diseased pods.Peanut is harvested at the high seed moisture content of about 30-50% or higher. Aflatoxin contamination causes by *Aspergillus flavus* is the major problems for human consumption of those peanut products and foods. This situation, which occurs in Magway district, Myanmar is similar to those, reported in many others countries.

Drying method of peanut kernels at the study area

Peanut crops harvested in the study area are sun dried on bare ground, tarpaulin and matting. Curing of peanuts was done for few days in the farms before drying and removing the seeds from the hulms, to remove some moisture content from the kernels. Drying is done in the sun. About 52.1 % and 30 % of farmers dried the peanuts for 3 days and 4 days. Only 17.9% of the farmers dried their pods for 7 days. Farmers should dry their nuts for 7 days with the duration from 7 to 12 days and should be aware of recommended methods of drying.To sell the higher weight of pods, most of the farmers do not dry properly and sell in the form of wet pods. In Magway district, only 17.9% farmers dried peanut pods until safe moisture content were obtained and sold peanuts to collectors in form of dry pods.In general, farmers stored their peanuts 1-7 days before selling to collectors. Collectors sold the peanuts to wholesalers and retailers in the form of dry pods and kernels. At each level, peanut kernels were stored in polypropylene bags.

In the study area, peanut pods from farmers are sundried on bamboo mats, Tarpaulin and bare ground or even roadside. About 54.3% of farmers dried the peanuts by spreading on thebare ground, 29.3 % used bamboo mat and tarpaulin was16.4% respectively. This increasedthe peanuts susceptibility to fungal contamination as spreading done on a hard surface causingthe injury to the peanuts. In addition, it increased cross contamination as a different crop may be spread on the same ground that was used for drying peanuts. Traditional peanuts drying techniques in developing countries like Myanmar involving filed and bare-ground drying are a major source of fungal contamination (Fig 2a &2b). Collectors and wholesalers, on the other hand, buy dried peanuts. About seventy five percent of the collectors and 70 % of the wholesalers dried again the peanuts. When the peanut kernels are not properly dried or when mold growth is visibly seen among the purchased peanuts, drying is done again.

Moisture content

The mean moisture content of peanut kernels of the delivery chain was considered to have safe moisture content. The mean moisture contents of peanut kernels from farmers, collectors and small wholesalers are presented in (Table 1, 2, and 3). Moisture content of the peanut kernels from the farmer's stores who dried for 3 days, 4 days, 7 days and 10 days are 11.51 %, 10.21%, 7.58%, and 7.22 % respectively. Moisture content of stored peanut kernels from farmers was similar to thosecollected from collectors and small wholesalers. Moisture content is the most important factor for affecting fungal growth for the stored peanuts. Peanuts are stable at 70% relative humidity between 7 - 9% moisture content, at which conditions fungal growth is arrested (ICAR 1987). Moisture content of pods and kernels are closely related with the drying process. Sundrying is the most critical postharvest handling procedure of peanuts, especially when the harvest coincides with the wet season. To get the higher weight, farmers from Myanmar do not dried properly and sold in the form of wet pods.

Sorting

Sorting is the final chance for the removal of broken or shriveled peanuts to reduce fungal contamination before processing. Approximately 95% of farmers, 89.3% of collectors, 60 % of wholesalers indicated that a change in peanut color as the obvious criteria used. The most common color changes indicated were brown, black and greenish. (Galvez 2003) showed that hand sorting has been shown to substantially reduce aflatoxin contamination in peanuts. All respondent from collectors and wholesalers level sorted the peanuts. The most commonly used storage material is the polypropylene bags. Up to 96.4% of farmers, 80 % of wholesalers and all respondents from collectors who had never had the word aflatoxin and are not aware of its effect on humans .The few (3.6%) who have heard of aflatoxins. In this study, about 38.6% of farmers re-dried their moldy peanut kernels and consumed food especially peanut oil. (Awuah 2009) studied that 40% of the farmers and 20% retailers converted spoiled nuts into other products for consumption.

Storage

About 38.6% of farmers stored the peanuts for 1to10 days and only 17% of farmers stored their peanuts for 2 months. The most commonly used storage material in study area is polypropylene bags under current condition. The fundamental reason why peanuts should be stored dry is to increase storability and in part, prevent growth of storage fungi.Storage structure commonly used in Myanmar is traditional

and may not maintain an even, cool and dry internal atmosphere; they do not provide the adequate production. Furthermore, a majority of farmers store peanuts in mixed structures. This has serious implications for cross-contamination and pest proliferation, thus increasing the risk of fungal contamination. (Fandohan 2005) reported that aflatoxin production is further increased during storage and improper handling practices.Lack of post-harvest handling practice is the most important problem in that study area. A clear outcome of the study is that farmers, collectors, wholesalers, processors and retailers were unaware the fungal contamination, and therefore they do not perceive fungal contamination as a problem in peanut production. Above bad post harvest drying and storage practice of farmers, collectors and wholesalers was correlated for fungal contamination.

Figure 2a: Drying of groundnuts on Tarpaulin

F igure 2b: Drying of groundnuts on bare ground

Frequencies of fungal isolation from peanut kernels

The fungi isolated from kernel samples during data collection are showed in (Table1, 2 & 3). The kernel samples of farmer's stores had higher frequency of isolation of *Aspergillus* spp. (mainly *Aspergillus* section*Flavi* and *Aspergillus* section *Orzyae* isolates) when compared with the collector and wholesaler's stores.In addition, *Aspergillus* spp. and *Aspergillus niger* were the most frequently isolated than *Aspergillus terreus* and *Penicillium citrinum*in this study. Among the species of fungi, *Aspergillus* spp.and *A.niger*were found as the commonly isolated species found in farmers, collectors and wholesalers 's stores. *Aspergillus* spp. was highest (40%) in its percentage of frequency, and followed by *A.niger* (24.17%) at the farmer's stores while these two fungus species were the next highest in their percentage of frequencies at small wholesaler's stores (Fig3).

On the other hand, *A.terreus* and *P.citrinum* were least in percentage of frequency in each store. In contrast to these, isolation frequencies of *Aspergillus* spp. or other fungi was not higher in any of collector's stores and small wholesaler's stores across the study area. The increase in the frequency of *Aspergillus* can also be explained by the fact that this genus is considered to be storage fungi, unlike *Fusarium* that exist in the field. In addition, *Aspergillus* spp. grow well at lower moisture content in contrast to other fungi as they require very high moisture content in the substrate for growth and mycotoxin (Hedayati 2010).

Pearson's correlation test between fungal presence of variables in peanut kernels showed moderate positive association between the presence of *Aspergillus* spp. and *A. niger* strains for farmer's stores (r = 0.486 and p =0.026), collector's stores (r =0.598 and p = 0.040), and a strong positive association between these fungi was

observed for wholesaler's stores (r = 0.865 and p = 0.0003). *Aspergillus* section *Nigri* species are considered an important competitor of *A.flavus (*Ashworth, Schroeder, and Langley 1965), which have similar nutritional requirements for growth and conidia germination and are often isolated in the same habitat (Griffin and Garren 1974).

The result showed that there was no correlation between the presence of *Aspergillus* spp. and *A.terreus* (p = 0.699) for farmer's stores and also reveled for collector's stores (p = 0.274) and wholesaler's stores (p = 0.671), respectively. In samples of farmer's stores positive correlation between *Aspergillus* spp. and *P.citrinum* (r =0.493;p = 0.023) was observed but however, no association between these fungi were observed for collector's stores and small wholesaler's stores. Furthermore, there was positive correlation between the presence of *A.niger* and *Aspergillus* spp. (r = 0.486;p = 0.026) and between *A. niger* and *A. terreus* (r = 0.571;p = 0.007) was observed but no correlation between *A.niger* and *P.citrinum* (p = 0.816) for farmer's stores. Asignificant correlation was observed between the presence of *A.niger* and *Aspergillus* spp. (r= 0.865;p = 0.0003) for wholesaler's stores but no significant correlation of these fungi was observedfor collector's stores. In addition, there were no correlations between the presence of *A.niger* and the remaining fungal genera isolated.

The moisture content of the stored peanut samples was less than 12 % at farm's store and less than 8% at collector and wholesaler's stores (Table 1, 2 & 3). The mean relative humidity and surrounding temperature of farmer's stores, collector's stores and wholesaler's stores are showed in (Table1, 2&3). (Nawar2008) showed that relative humidity plays a vital role in the development and spread of fungal contamination. The report of different fungal contamination rate by different investigators may be a result of adverse pre-harvest conditions of temperature and humidity in the field and improper post-harvest handling and storage (Nakai 2008).

The highest mean contamination of kernels with *Aspergillus* spp. was found at each storehouse. Statistical analysis showed a significant correlation (p< 0.05) of *Aspergillus* spp. with moisture content of kernels for farm and collector' stores but no significant correlation was observed for wholesaler's stores. In addition, a significant correlation of the same fungus with temperature for farm site was found but no significant correlation was observed for collector and wholesaler's stores while no significant correlation was observed for *Aspergillus* spp. with relative humidity for farm stores but a significant correlation was observed for wholesaler's stores (Table 4).

Figure 3: Overall frequency (%) of fungal contamination in peanut kernel samples in farmer's stores, collector's stores and wholesaler's stores

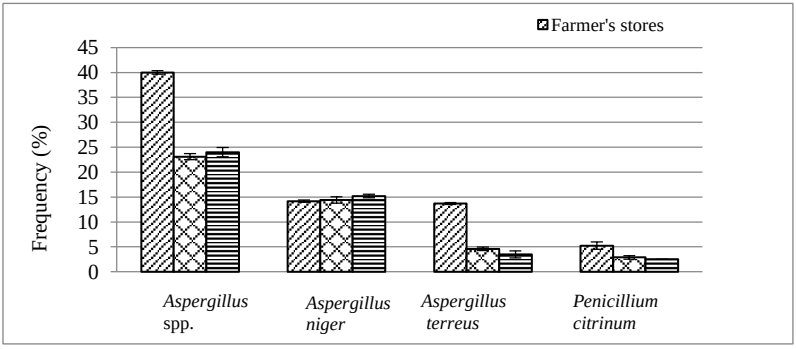


Table 1: Frequency of *A.niger*, *A.terreus*, *Aspergillus* spp. and *P.citrinum*, abiotic factors (temperature, relative humidity), and moisture content measured in farm stores

Storage time	Fungal frequencies (%)				Tempera-ture (°C)	RH (%)	Moisture Content (%)
	Aspergillus spp.	A. niger	A. terreus	P. citrinum	Mean	Mean	Mean
3 days of storage	57	33.3	18	5.7	33.6	54.7	11.51
4 days of storage	45.5	21.7	12.5	4.8	34.3	58	10.21
7 days of storage	30.3	22.3	17.4	3.3	33	53	7.58
10 days of storage	32.8	27.1	20	1.7	33.5	57.3	7.22
30 days of storage	42.6	17.3	9.8	9.1	34	53.8	7.51
45 days of storage	40.7	31	11	7.5	33.1	55.8	7.22
60 days of storage	31.7	17.3	8	2.3	32.8	58.1	7.78

Table 2: Frequency of *A.niger*, *A.terreus*, *Aspergillus* spp. and *P.citrinum*, abiotic factors (temperature, relative humidity), and moisture content measured in collector's stores

Collection	Fungal frequencies (%)				Tempera-ture (°C)	RH (%)	Moisture Content (%)
	Aspergillus spp.	A. niger	A. terreus	P. citrinum	Mean	Mean	Mean
15 days of storage	11.7	10.3	4.3	2.3	32.6	54	7.2
30 days of storage	25	20.7	6.6	1.7	35.2	51.2	7.52
45 days of storage	33.3	12.5	5.3	2.7	33.8	53	7.62
60 days of storage	23	15.3	1.3	1.7	33.2	62.2	7.4

Table 3: Frequency of *A.niger*, *A.terreus*, *Aspergillus* spp. and *P.citrinum*, abiotic factors (temperature, relative humidity), and moisture content measured in wholesaler's stores

Collection	Fungal frequencies (%)				Tempera-ture (°C)	RH (%)	Moisture Content (%)
	Aspergillus spp.	A. niger	A. terreus	P. citrinum	Mean	Mean	Mean
15 days of storage	23.3	14.2	5.7	1.7	33	51.2	7.62
30 days of storage	22.8	17.3	3.3	1.8	35.2	54.6	7.78
45 days of storage	39.2	19.3	1.5	2.5	32	55	7.86
60 days of storage	11.7	11	1.7	2.2	32.4	63.8	7.84

Table 4: Probability values for *Aspergillus* spp. isolated from kernels of farmer, collector and wholesaler's storehouses

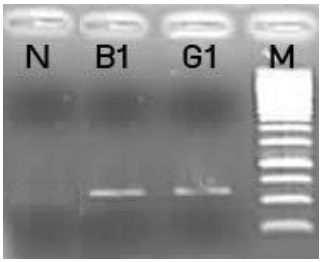
Collection	Variables		
	Moisture content (%)	Temperature (°C)	Relative Humidity (%)
Farmer's stores	<0.0001	0.0074	0.5566
Collector's stores	<0.0001	0.064	0.6879
Wholesaler's stores	0.6245	0.5014	0.0473

Table5: Primer sets and corresponding amplification targets

Target gene	Primer	Primer DNA sequence	PCR product size
ITS	ITS5	5' GGAAGTAAAAGTCGTAACAAGG 3'	601
	ITS4	5' TCCTCCGCTTATTGATATGC 3'	
	ITS5	5' GGAAGTAAAAGTCGTAACAAGG 3'	548
	ITS4	5' TCCTCCGCTTATTGATATGC 3'	
β-Tubulin	Bt2a	5' GGTAACCAAATCGGTGCTGCTTC 3'	520
	Bt2b	5' ACCCTCAGTGTAGTGACCCTTGGC 3'	
	Bt2a	5' GGTAACCAAATCGGTGCTGCTTC 3'	468
	Bt2b	5' ACCCTCAGTGTAGTGACCCTTGGC 3'	

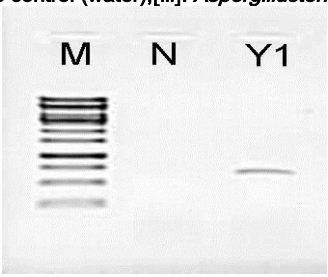
Identification of Aspergillus species and Penicillium.citrinum

Figure 4a: Gel electrophoresis photo showing DNA portions of *Aspergillus* isolates result from PCR reactions primers by ITS5/ITS4 and Bt2a/Bt2b fungal primer, [i]: Negative control (water), [ii]: *Aspergillus niger,* [iii]: *Aspergillus* spp.and [iv]: 1kbp DNA marker



i ii iii iv

Figure 4b: Gel electrophoresis photo showing DNA portions of *Aspergillus* isolate result from PCR reactions primers by ITS5/ITS4 universal fungal primer,[i]:1kbp DNA marker[ii]: Negative control (water),[iii]: *Aspergillusterreus*



i ii iii

Briefly, Fungal DNA was extracted from the pure isolates and Polymerase Chain Reaction (PCR) was performed to detect beta tubulin genes and interal transcribe spacer genes. PCR products were visualized in gel electrophoresis for the presence of internal transcribe spacer and beta tubulin genes (Fig.4a & 4b). DNA sequence of those PCR products were identified in both directions on the automated DNA sequencer (Macrogen Inc., Korea). Resulting DNA sequence were checked in GenBank using BLAST (Basic Local Alignment Tool) to match the DNA sequence of the existing fungal species from the GenBank database.

A total of 75 isolates of *Aspergillus* species and 10 isolates of *Penicillium* species were determined from the peanut kernel samples collected from Magway district, Myanmar. Three different *Aspergillus* spp. and one *Penicillium* species were found to be associated with peanut kernel samples. Molecular size of the DNA of fungal species were determined by the fluorescence intensity and comparison of the distance travelled with that of the molecular weight of marker standard as measured using gel electrophoresis, and as shown in (Fig 4a &4b). However, the data

indicated that the DNA fragment in lane ii *A. niger* compared to lane iii *Aspergillus* spp. has relatively distinct molecular sizes of (601) bps and (520) bps (Fig.4a) and the DNA fragment in Lane iii *Aspergillus terreus* (548) bps (Fig.4b). The distinct molecular size of *Penicillium citrinum* was (468) bps not shown in figure.

Identification of fungi by molecular means is considered the most reliable over conventional method. Though, it is expensive, labour and time intensive, it has become the most common tool for rapid identification of *A.niger*, *Aspergillus* spp. and other types of fungi. The species of fungi identified are not morphologically and molecularly similar; however, they can be identified further as a variety if closely related species.Suggestions made by (Martinez-Culebras and Ramon 2007,Varga 2011 and El khoury 2011) using beta-tubulin genes can be adopted in developing a differential relationship between closely related species of fungi like these.Molecular methods have been extensively useful in the identification of these *Aspergillus* species and several techniques such as random amplified polymorphic DNA analysis, DNA sequencing (Paterson and Russell2006) and specific diagnostic PCR primers (Nicholson 1996) have been established for their systematic studies. Therefore, molecular characterization has also been carried out in this study, to identify the frequently isolated species of *Aspergillus* and *Penicillium*.

Identification of the four chosen isolates (G_1, B_1, Y_1, and B_2) was based on the ITS and Beta-Tubulin regions. Thus, a set of primers (ITS5 and ITS4) and (bt2a and bt2b) was used to amplify the rDNA region (Table 5). Theblast data showed that the isolates $G_1$was related to *A. flavus*strain AG14 beta-tubulin gene partial cdswith a similarity 100 % and *A. oryzae* strain USME 09 beta-tubulin gene partial cds100 %, whereas the isolate B_1 matched CBS 132413 *A. niger*, N3: rDNA sequences ITS with a 100 % similarity. However, the isolate Y_1 was more closely related to *A. terreus* isolate 3.1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNG gene, partial sequence with a 99% similarity whereas the isolate B_2 was related to *Penicillium citrinum*strain 139P beta-tubulin gene, partial sequence with a similarity 100%.

Conclusion

The present study indicated that the factors of the effect of postharvest management of the study area and the factors which are the associated of fungal contamination of peanut kernels in Myanmar. In addition, the result showed that farmer's practice of drying, storage and handling were important for the growth of the fungus. Traditional peanuts drying techniques in developing countries like Myanmar involving filed and bare-ground drying are a major source of fungal contamination. Lack of post-harvest handling practice is the most important problem in that study area. A clear outcome of the study is that farmers, collectors, and wholesalers were unaware the fungal contamination, and therefore they do not perceive fungal contamination as a problem in peanut production. Above bad post harvest drying and storage practice of farmers, collectors and wholesalers was correlated for fungal contamination. The presence of fungi can damage grains. Furthermore, if the strains were toxigenic, mycotoxin production is an imminent risk. In this study, despite the high frequency of isolation of *Aspergillus* spp. and *Aspergillus niger*, the moisture content of the peanut kernel samples (< 11%) are not sufficient for growth fungus . Most of the samples contaminated with *Aspergillus* spp. and the study highlight the need to improve the postharvest handling and good storage practice to prevent fungal contamination during storage of peanut kernels in Myanmar. The specific primer with PCR identified *A.niger*, *A.terreus*, *Aspergillus* spp. and *P.citrinum* and this molecular method is very useful for the identification of fungi.

References

Afolabi, C.G., Bandyopadhyay, R., Leslie, J.F. and Ekpo, E.J.A. 2006. Effect of sorting on incidence and occurrence of fumonisin and fumonisin-producing *Fusarium* species on maize (*Zea mays* L.) in Nigeria. *Journal of Food Protection* 69: 2019-2023.
Ashworth, L. J., Jr., Schroeder, H. W. and Langley, B. C. 1965. Aflatoxins: Environmental factors governing occurrence in Spanish peanuts. Science, 148, 1228–1229.
Awuah, R. T., S. C. Fialor, A. D. Binns and C. M. Jolly. 2009. "Factors Affecting Decision to Sort Groundnuts Along the Marketing Chain in Ghana." Peanut Science 36: 68-76.
Awuah, R.T. and Kpodo, K.A. 1996. High incidence of *Aspergillus flavus* and aflatoxins in stored groundnut in Ghana and the use of a microbial assay to assess the inhibitory effect of plant extracts on aflatoxin synthesis. Mycopathologia 134: 109-114.
Bulaong, S.S.P. and Dharmaputra, O.S. 2002. Fungal population, aflatoxin and free fatty acid contents of peanuts packed in different bag types. Biotropia 19: 1-25.
Davison, J. L., Whitaker, T. B. and Dickens, J. W. 1982. Grading, cleaning, storage, shelling, and marketing of peanuts in the United States. In H. E. Pattee, & C. T. Young (eds.), Peanut science and technology (pp. 571–623). Yoakum: American Peanut Research and Education Society.
El khoury, A., Atoui, A., Rizk, T., Lteif, R., Kallassy, M. and Lebrihi, A.2011. Differentiation between *Aspergillus flavus* and *Aspergillus parasiticus* from pure culture and aflatoxin-contaminated grapes using PCR-RFLP analysis of aflR-aflJ intergenic spacer. *J. Food Sci*. 76, 247-253.
Ezekiel, C. N., Odebode, A. C. and Fapohunda, S. O. 2008. Zearalenone Production by naturally occurring *Fusarium* sp. on maize, wheat and soybeans from Nigeria. *Journal of Biology and Environment Science, 6*, 77-82.
Fandohan, P., Gnonlonfin, B., Hell, K., Marasas, W.F.O. and Wingfield, M.J. 2005. Natural occurrence of *Fusarium* and subsequent fumonisin contamination in pre-harvest and stored maize in Benin, West Africa. *International Journal of Food Microbiology* 99: 173-183.
Galvez, F, C, F., Francisco, M, L, D., Villarino, B, J., Lustre, A, O. and Resurrection, A, V, A. 2003. Manual sorting to eliminate aflatoxin from peanuts. *Journal of Food Protection*, 66, 1879-1884
Gherbawy, Y. and Voigt, K. 2010. Molecular Identification of Fungi. Springer.
Griffin, G. J. and Garren, K. H. 1974. Population level of *Aspergillus flavus* and *A. niger* group in Virginia peanut field soils. Phytopathology, 64, 322–325.
Hedayati MT, Pasqualotto AC, Warn PA, Bowyer P and Denning DW. 2007. *Aspergillus flavus*: human pathogen, allergen and mycotoxin producer. Microbiology 153:1677-1692.
Hell, K. and Mutegi, C. 2011. Aflatoxin control and prevention strategies in key crops of sub-Saharan Africa. *African Journal of Microbiology Research* 5: 459-466.
Hell, K., Cardwell, K.F., Setamou, M. and Poehling, H.M. 2000. The influence of storage practices on aflatoxin contamination in maize in four agroecological zones of Benin, West Africa. *Journal of Stored Products Research* 36: 365-382.
ICAR. 1987. Aflatoxin in Groundnut-technologies for Better Crops. Publications and Information Division. Indian Council of Agricultural Research, New Dehli, India.
Kaaya, N.A. and Warren, H.L. 2005. A review of past and present research on aflatoxin in Uganda. *AfricanJournal of Food Agriculture and Nutritional Development* 5: 1-17.
Kimatu, J.N., McConchie, R., Xie, X. and Nguluu S.N. 2012. The significant role of post-harvest management in farm management, aflatoxin mitigation and food security in Sub-Saharan Africa. *Greener Journal of Agricultural Sciences* 2: 279-288.
Klich, M.A., Tang, S., and Denning, D.W. 2009. Aflatoxin and ochratoxin production by *Aspergillus* species under *ex vivo* conditions. Mycopathologia 168: 185-191.
Martinez-Culebras, P.V., and Ramon, D. 2007. An ITS-RFLP method to identify black Aspergillus isolates responsible for OTA contamination in grapes and wine. *Int. J. Food Microbiol*. 113, 147-153.
Nakai V.K, Rocha LO, Gonçalez E, Fonseca H, Ortega EMM, and Correa B. 2008.Distribution

of fungi and aflatoxins in a stored peanut variety. Food Chem. 106:285-290.
Nawar L.S. 2008. Prevention and control of fungi contaminated stored pistachio nuts imported to Saudi Arabia. Saudi J. Biol. Sci. 15:105- 112.
Nicholson, P., Lees, A.K., Maurin, N., Parry, D.W., and Rezanoor, H.N. 1996. Development of a PCR assay to identify and quantify Microdochium nivale var. nivale and Microdochium nivale var. majus in wheat. Physiol. Mol. Plant Pathol 48, 257-271.
Park, D.L. 2002. Effect of processing on aflatoxin. Advances in Experimental Medicine and Biology 504: 173-179.
Paterson, M.and Russell, R. 2006. Identification and quantification of mycotoxi-genic fungi by PCR. Process Biochem. 41 (7), 1467-1474.
Reddy, K. R. N., Reddy, C. S. and Muralidharan, K. 2009. Potential of botanicals and biocontrol agents on growth and aflatoxin production by *Aspergillus flavus* infecting rice grains. *Food Control*, *20*, 173-178.
Udoh, J.M., Cardwell, K.F. and Iltotun, T. 2000. Storage structures and aflatoxin content of maize in five agroecological zones of Nigeria. *Journal of Stored Products Research* 36: 187-201.
Varga, J., Frisvad, J.C. and Samson, R.A. 2011. Two new aflatoxin producing species, and an overview of *Aspergillus* section *Flavi*. Stud. Mycol. 30, 57-80.

Estimation of Antioxidant Activity, Total Phenol and Flavonoid Content of Some Myanmar Medicinal Plants

KHIN KHIN WIN AUNG, MYAT CHAW SU, GADAE MAUNG MAUNG, YU YU NWE OO, KAY THI KYAW, MYINT MYINT KHAING, MAR MAR MYINT, PHU PHU WIN

Pharmaceutical Research Department, Chemical Technology Research Centre, Department of Research and Innovation, Ministry of Science and Technology

Abstract

The purposes of this research are to promote the local traditional medicine products with the scientific experimental results by evaluation of the antioxidant activity of Myanma natural plants and to produce the effective alternative medicine. Seven plants were selected to determine the antioxidant activity by of 2,2-Diphenyl 1-picryl hydrazyl (DPPH) free radical scavenging assay in this research. Preliminary phytochemical test for seven plant samples were made to identify types of compounds. According to the results, cyanogenic glycoside was not found in these samples. Therefore, they have potential safety for further research. These plant samples were extracted with methanol to evaluate the antioxidant activity. The results of antioxidant activity of *Garcinia magostana* L. (peel) 92.09%, *Glycyrrhiza glabra* L. (stem) 85.96% and *Curcuma longa* L. (rhizome) 80.05% in highest concentration of 100μg/ml were showed more than 80% than the other plants. IC_{50} value of *Garcinia magostana L.* was (0.08 μg/ml) less than 50 so that it was the highest antioxidant activity among these plants. Phenol and flavonoid compounds in the plant were related with the antioxidant activity. Therefore, content of total phenol in these three active plant samples was determined by using Folin-Ciocalteu phenol reagent colorimetric (ISO 14502-1:2005-E) method to know the total phenol content. *G. magostana* (8.65%), *G. glabra* (3.38%) and *C. longa* (4.50%) of total phenol content were recorded individually. Among these plants, *G. magostana* contained the highest amount of phenol content followed by the order *C. longa* and *G. glabra*. Total flavonoid content was estimated by aluminium chloride colorimetric method described by Chang et.al. (2002). The results of these samples were ranged from 0.80% to 6.36%. *C. longa* contained the highest amount of flavonoid content followed by the order *G. magostana* and *G. glabra*. This research work can be benefit in the role of traditional medicine by determination of antioxidant activity from the plant extract of some Myanmar medicinal plants.

Introduction

Human being has energy by activities of eating, respiration and free radicals come out as byproducts in the body. Free radical is an atom with at least one unpaired electron and cannot stand itself. It can also be emitted by environmental factors which are stress, smoking, metabolism in the body, pollutions, chemicals, ultraviolet light and radiations etc. As free radicals combine with electron of other atoms and induce the oxidation, caused to raise the amount of free radicals. Although free radicals can attack bacteria, virus and keep our brains sharp, they will break DNA (deoxyribonucleic acid) that control genes, cells and organs by oxidation if they increase than normal. Finally, premature aging, cardiovascular disease, inflammation, stroke and cancer disease can be caused. Moreover, human need to eat and know about rich in antioxidant food to reduce free radicals production that creates the health problem.

Plant sourced antioxidants like vitamin C, vitamin E, carotene, phenolic acid etc. have been recognized as having the potential to reduce disease risk.[1] Not only antioxidants can prevent the cancer disease and numerous other health problems but also they can support the longevity and rejuvenation so for that people eat them. Nowadays, not only western medicine but also alternative medicine is used to solve health problem. The medicinal plants are normally used in traditional medicine for various illness or diseases. The traditional medicines are better needed to know their antioxidant activities of some medicinal plants.

Flavonoids are a large group of phenolic plant constituents. The antioxidant activity of phenolic compounds in plants is mainly due to their redox properties and chemical structure, which can play an important role in neutralizing free radicals, chelating transitional metals and quenching triplet oxygen by delocalization or decomposing peroxides.[2] Flavonoids in plants also act as antioxidant, antimicrobials, photoreceptors, visual attractors, feeding repellants, and for light screening.[3] Flavonoids preparation has been used to treat disorders of peripheral circulation for over 40 years.[4] Regular consumption of flavonoids and phenol may contribute to free radical scavenging, anti-inflammatory, coronary heart disease and inhibition of LDL oxidation.[5] The antioixidant activity of plants has significantly correlated with total phenol content.[6] Some studies have shown that the dietary polyphenolic constituents derived from plants are more effective antioxidants *in vitro* than vitamin E or C, and thus might contribute significantly to the protective effects *in vivo*.[7]

The selected plants are *Garcinia magostana* L.(peel), *Curcuma longa* L. (rhizome), *Glycyrrhiza glabra* L.(stem), *Andrographis paniculata* Nees (the whole plant), *Carrica papaya* L.(leave), *Cuscuta reflexa* Roxb. (stem) and *Dolichandrone spathaceae* (L.f) K. Schum (flower). These plants were the use of traditional medicines formulation in Myanmar and they were selected for this research.

Medicinal uses and chemical constituents of selected medicinal plants:

Garcinia magostana: It was used waste rind as a traditional medicine for the treatment of abdominal pain, diarrhea, dysentery, infected wound, suppuration and chronic ulcer, antioxidant, anti-allergies, inflammations, antivirus, anti-bacteria and anticancer. It contains cycloartenol, β-sitosterol, friedin, mangiferadiol, mangiferolic acid, gartanin and xanthone compound etc.

Curcuma longa: It has an antioxidant activity and can prevent blood flow to cancer cells. Turmeric has been used for centuries to treat stomach problems, anti-inflammatory, antioxidant, antitumour, antiseptic properties and help stimulate bile production, which prevents gallstone formation, alzheimer's disease, arthritis and rheumatoid arthritis. It contains sespqiterpenes, p-tolylmethyl carbinol, curcumin, protein, carbohydrates and carotene etc.

Glycyrrhiza glabra: It has tonic, expectorant, gastric ulcers and the extract is reported to be useful also in the treatment of Addision's disease. Root contains glycyrrhizin, asparagin, sugar, starch, acid resin, phosphoric, sulphoric and malic acids. Glycyrrhiza content varies in different extracts from 12-24%, glucose 3.8%, sucrose 2.4-6.5% starch 30%, asparagine, bitter principles, resin (2-4%) and a volatile oil (0.03-0.035%).

Andrographis paniculata: It is a powerful bitter tonic and used for fever, dysentery, diarrhea diseases. The major components of the leaves contain andrographolides.

Carrica papaya: The leaves have anti-malarial, anti-plasmodial properties and can treat heart conditions, cancer, stroke, dengue fever, whooping cough and other respiratory ailments. It contains carpanie, nicotinine, myosmine, carotenoids, rutin, carpoaside, luic acid, flavonoid, riboflavine, ascorbic acid, xanthopylls and papain.

Cuscuta reflexa: Stems in decoction are useful in constipation, flatulence, liver complaints and bilious affection and are also externally used against itch and other skin diseases. It contains scoparoe, melanettin, quercetin, hyperoside, dulcitol, leutolin, cuscutin, cuscutalin, bergenin, kaempferol, amarbelin and β-sitosterol.
Dolichandrone spathaceae: People has been using flowers for hepatitis disease for local uses in Myanmar. It contains triterpene and saponin etc. [8,9,10,11,12]
The aims of this study are to determine the antioxidant potential of the selected plants which are normally used in Myanmar traditional medicine and to examine their total phenol and flavonoid contents.

Materials and Methods

Plant materials
G. magostana L., *G. glabra* L.(stem), *D. spathaceae* (L.f) K. Schum, *C. longa* L., were purchased from local herbal market and other plants were collected from various locality in Yangon, Myanmar. The selected plant samples were identified by an expert botanist.
Equipments
Beakers, cornical flasks, graduated cylinders, volumetric flasks, pasteur pipettes, test tubes, percolator and micropipette.
Instruments
UV/VIS/NIR Spectrometer (PerkinElmer Lamda 950), voltex mixer (VM 200, Thaiwan), water bath (LWB 111D, Labtech, Korea), analytical balance (AX 200, Shimadzu), oven (Chongqing, China), shaker (Auther thomas, USA), rotary evaporator (HS-3001-N, Korea) and centrifuge (DSC-301SD-SH, Thaiwan).
Chemicals
Methanol, ethanol, 2,2-diphenyl 1- picryl hydrozyl (DPPH), L-Ascorbic acid, Folin-Ciocalteu phenol reagent, aluminium chloride, gallic acid, quercetin and potassium acetate were purchased from Sigma Chemicals (St. Louris, USA) and sodium carbonate (BDH chemicals Ltd. England) were used in this research. Other chemical were purchased from local chemical market.

Collection and Preparation of Plant Samples

Collected plant parts were cleaned with water and air-dried in shade at room temperature. After drying, they were cut into small pieces and ground to fine powder using grinder.

Preparation of Plant Extract

5 g of each plant samples were dissolved in methanol and was shaken on mechanical shaker at room temperature for successive 2 days. The methanol extracts were filtered through filter paper and the filtrate were separately concentrated to dryness in using a rotary evaporator to remove the solvents.

Determination of Antioxidant Activity

In this study *in vitro* free radical scavenging assay was applied for reducing ability of DPPH free radical. The deep purple color of freshly prepared DPPH solution changed to the colorless or fade if treated with the solution containing antioxidant herbal extract when the maximum absorption at 517 nm. DPPH scavenging activity described by Neha, J. et. al. (2011) was adapted.[13] 0.004% of DPPH (2,2-Diphenyl 1-picryl hydrazyl) solution was freshly prepared with methanol before the measurement. Each plant extract 0.1 g was dissolved in 5 ml methanol and diluted to 20 μg/ml, 40 μg/ml, 60 μg/ml, 80 μg/ml and 100μg/ml respectively. Two mililiter of DPPH reagent was separately reacted with prepared plant sample solution (2 ml).

The reaction mixture was incubated in the dark room for 30 min at room temperature. All the tests and analysis were performed in triplicates and averaged. L-ascorbic acid was used for standard control for the DPPH scavenging activity. IC_{50} value for DPPH scavenging activity of plant extracts are shown in Table 1. The percentage of DPPH scavenging activity was calculated by the following formula.

$$\text{DPPH scavenging activity (\%)} = \frac{\text{Absorbance control - Absorbance sample x 100}}{\text{Absorbance control}}$$

where, Absorbance control was Absorbance of DPPH and methanol, Absorbance sample was Absorbance of extract and methanol.
If DPPH free radical obtain electron, the absorbance of plant extract will decrease and the antioxidant activity will also increase depending on concentrations.

Determination of Total Phenol Content

Total phenol content of plant samples were determined by using Folin-Ciocalteu phenol reagent colorimetric method (ISO 14502-1:2005-E).[14] 0.2 g of plant sample was extracted with 70% methanol at 70°C for 30 minute. The mixture was vigorously shake on voltex mixer. It was centrifuged at 3500 rpm for 10 minutes and was decanted the supernatant. This process was repeated for complete extraction of the sample and combined the extracts. The extract volume was made up to 10 ml with cold 70% methanol and mixed thoroughly the contents. The extract was stored at 4°C and it was used within 24 hrs. The standard Gallic acid solution was also prepared the various concentrations for 10 μg/ml, 20 μg/ml, 30 μg/ml, 40 μg/ml and 50 μg/ml. These solutions were freshly prepared on the day of use. 7.5% sodium carbonate solution and 10% Folin-Ciocalteu phenol reagent were also prepared. After preparation of reagents and plant samples, diluted plant extract 1 ml was reacted with 5 ml of diluted 10% Folin-Ciocalteu phenol reagent. After 3-8 min, 4 ml of 7.5% sodium carbonate solution was added to the mixture of each plant sample solution. After incubation for 1 hr at room temperature, the optical density of the tested solution was measured with UV/VIS/NIR spectrometer (Lamda 950) at 765 nm. The optical density of reagent blank was 0.007. Gallic acid was used to obtain the standard calibration curve. The total phenolic content was expressed as gallic acid equivalents (GAE). All samples were tested in duplicate and two separate determinations were carried out. Total phenol contents of plant samples were expressed as a percentage by mass on a sample dry matter basis was given by the formula.

$$\text{TPC (\%)} = \frac{(D_{sample} - D_{intercept}) \times V_{sample} \times d \times 100}{S_{std} \times m_{sample} \times 10000 \times W_{DM,sample}}$$

where, TPC was totoal phenol content, D_{sample} was the optical density obtained for the sample test solution, $D_{intercept}$ was the optical density at the point the best-fit linear calibration line intercepts the y axis, V_{sample} was the sample extraction volume, in milliliters, d was the dilution factor used prior to the colorimetric determination, S_{std} was the slope obtained from the best-fit linear calibration, m_{sample} was the mass, in grams, of the sample test portion, $W_{DM,sample}$ was the dry matter content, expressed as a mass fraction in %, of the test sample determined in accordance with ISO-1752 : 1980.

Determination of Dry Matter Content

To know the total phenol content in plant sample, dry matter content was determined by using ISO-1572:1980 (E) method and calculated in the following formula.[15]

$$\frac{M_1 \times 100}{M_0}$$

where, M_1 was the initial mass (g) of the test portion, M_0 was the mass (g) of the dried portion.

Determination of Total Flavonoid Content

Total flavonoid content was determined by using aluminium chloride colorimetric method described in Chang et. al., 2002.[16]. Quercetin was used to make standard calibration curve. Each plant extract 1 g from raw dry sample was dissolved in 25 ml methanol. The different concentrations of qurcetin 10 μg/ml, 20 μg/ml, 30 μg/ml, 40 μg/ml and 50 μg/ml were prepared from stock solution of 1 mg/ml. 1ml of diluted standard solution, 3 ml of methanol, 0.2 ml of 10% aluminium chloride, 0.2 ml of 1M potassium acetate and 5.6 ml of distilled water were added to each concentration of standard solution. The reaction mixture was incubated at room temperature for 30 minutes and measured with a UV/VIS/NIR spectrometer at 415 nm to obtain the optical density of different concentration of standard. Similarly, each plant extract solution (1ml) was used instead of diluted quercetin standard solution in the above procedure. The total flavonoid content of standard and sample were calculated in the following formula.

$$TFC\ (\%) = R \times DF \times V \times 100 / V$$

where, TFC was total flavonoid content, R was results obtained from the standard curve, DF was dilution factor, V was volume of stock solution, 100 was for 100 g dried plant, W was weight of plant used experiment in mg.

Result and Disscussion

In this research work, preliminary phytochemical tests had been done for seven plants to identify the type of compounds for antioxidant activity. According to the results, they did not contain cyanogenic glycoside. Therefore, all plant samples have potential safety for further research. To know the antioxidant activity, DPPH free radical scavenging activity was carried out. DPPH radical scavenging test is based on the exchange of hydrogen atoms between the antioxidant and the stable DPPH free radical. DPPH is a stable free radical at room temperature which accepts an electron or hydrogen radical to from a stable diamagnetic molecule. DPPH radical is reduced to the corresponding hydrazine, a color change of the solution from violet to yellow is observed and that is monitored spectrophotometrically. More reduction of DPPH radical is related to the high scavenging activity of the particular extract. [17]

The results of antioxidant activity of *G. magostana* (peel) 92.09%, *C. longa* (rhizome) 80.05% and *G. glabra* (root) 85.96% were recorded. The antioxidant activity of these extracts showed 80% and above than the other plants which showed the moderate activity as shown in Table (1). *G. magostana* can be compared with antioxidant activity of standard ascorbic acid. IC_{50} value of these three plants was 0.08 μg/ml, 0.1μg/ml and 0.13 μg/ml respectively so that *G. magostana* was very strong antioxidant activity among plants as shown in Table (2) and Figure (1). The peel of *G. magostana* had the highest antioxidant activity and it was mainly used in Myanmar traditional medicine to cure dysentery. Some Myanmar authors described about the medicinal uses of *G. magostana* fruit and the effective peel. Moreover, it was reported that *G. magostana* possessed several

biological and pharmacological properties such as antihistamine, antifungal, antibacterial, anti HIV protease and induction of apoptosis in cancer cell lines.[18] The oil of *G. glabra* was showed antioxidant activity by *in vitro* DPPH model in dose dependent manner and also showed antifungal, antiulcer and antiaflatoxigenic activity.[19] A group of neolignan lipid ester and phenolic compound isolated from the roots and stolons of liquorice (*G. glabra*) were found to have chemopreventive properties. Of these compounds, hispaglabridin B, isoliquiritigenin, and paratocarpin B were found to be the most potent antioxidant agents.[20] There are several studied that these beneficial properties of *C. longa* L. have been associated to the antioxidant activity.[21,22,23] Curcuminoids (curcumin, demethoxycurcumin and bisdemethoxycurcumin), the phenolic yellowish pigments of *C. longa* L. have been suggested to have antioxidative, anticarcinogenic, anti-inflammatory and hypocholesterolemic activities[24]. Furthermore, natural products of plant origin have been proposed as a potential source of natural antioxidants with strong activity. It is mainly due to the presence of phenolic compound like flavonoids, phenols, flavonols and proanthocyanidins.[25]

Total phenol contents of three active antioxidant plant samples determined by Folin-Ciocalteu phenol reagent colorimetric method (ISO 14502-1:2005-E) were reported as gallic acid equivalents as shown in Figure (2). Total phenol contents of *G. magostana* (8.65%), *C. longa* (4.50%) and *G. glabra* (3.38%) were recorded as shown in Table (3). Among these plants, *G. magostana* contained the highest amount of phenol content followed by the order *C. longa* and *G. glabra*.

Total flavonoid contents in these plants samples were ranged from 0.08% to 6.36 % and determined by aluminium chloride colorimetric method. Quercetin was used as calibration curve standard as shown in Figure (3). *C. longa* was contained the highest amount of flavonoid content followed by the order *G. magostana* and *G. glabra*. Scientists presented the flavonoid, tannin, phenolic compound were related to the antioxidant activity.[26,27,28,29]

Table 1: Antioxidant Activity of and Selected Medicinal Plants and Standard Control

No	Scientific Name	Antioxidant Activity (%)				
		100 µg/ml	80 µg/ml	60 µg/ml	40 µg/ml	20 µg/ml
1	G. mangostana L.	92.09	91.86	90.16	87.15	78.43
2	C. longa L.	80.00	78.00	75.00	68.00	61.00
3	G. glabra L.	85.96	84.46	81.86	74.79	63.90
4	A. paniculata Nees	59.24	57.20	56.24	53.62	51.03
5	C. papaya L.	63.52	60.73	60.16	55.08	50.34
6	C. reflexa Roxb.	59.41	57.56	54.29	50.80	49.79
7	D. spathacea K.	56.65	51.37	50.73	49.51	48.17
8	Ascorbic Acid	98.02	97.89	97.84	97.77	97.72

Table 2: IC_{50} value of Selected Medicinal Plants and Standard Control

Samples	Family	English Name	IC50 (mg/ml)
G. magostana	Guttiferae	Mangosteen	0.08
C. longa	Zingiberaceae	Turmeric	0.1
G. glabra	Papilionaceae	Sweet root	0.13
A. paniculata	Acanthacae	King of Bitter	3.16
C. papaya	Caricaceae	Papaya	12.94
C. reflexa	Convolvulaceae	Dodder plant	24.8
D. spathaceae	Bignoniaceae	Mangrove trumpet tree	51
L-Ascorbic acid	-	-	0.02

Figure 1.: IC_{50} value of Selected Medicinal Plants and Standard Control

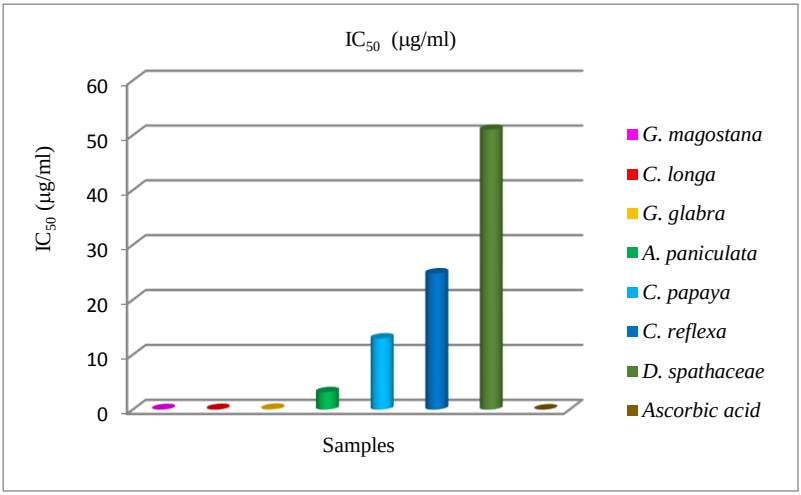


Figure 2: Gallic Acid Calibration Curve

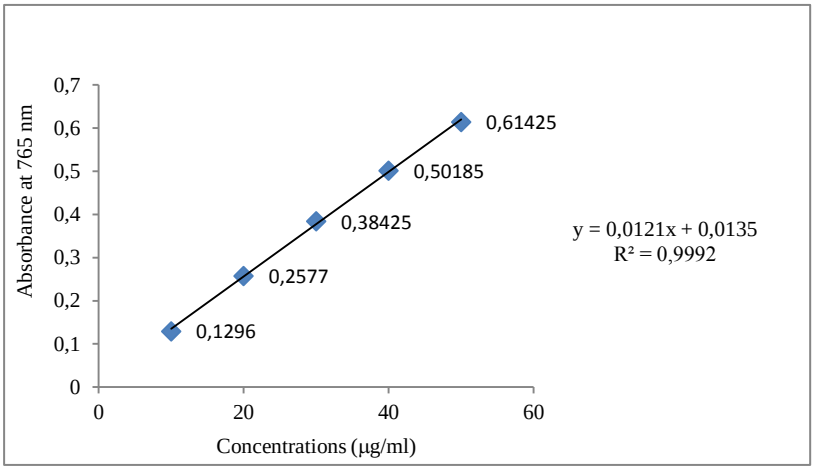


Figure 3: Quercetin Calibration Curve

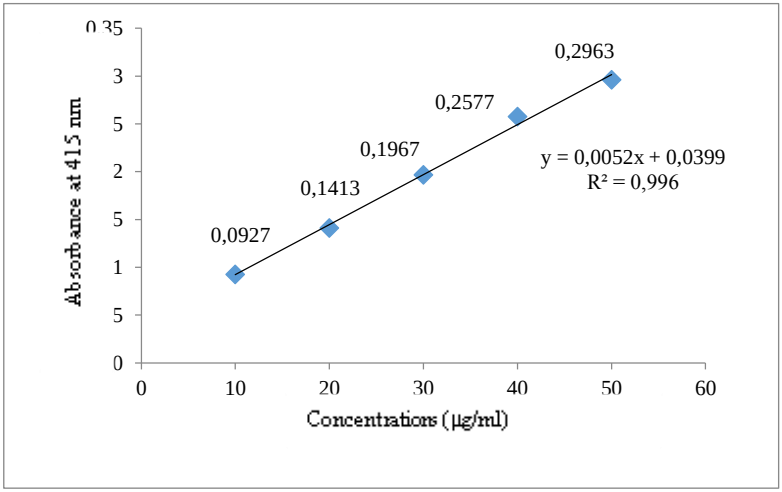


Table 3: Total Phenol Content and Flavonoid Content of Plant Samples

Plant Samples	Total Phenol Content (%)	Total Flavonoid Content (%)
G. magostana L.	8.65	1.82
C. longa L.	4.50	6.36
G. glabra L.	3.38	0.80

Conclusion

Most plants have many biological effects as antioxidant activity included phenolic and flavonoid compounds. As the selected plants have active potential, this research was a fundamental work for further research. *G. magostana*, *G. glabra* and *C. longa* showed the highest radical scavenging activity among these seven plants. Nowadays, antioxidant supplements and medicines are more popular for anti-aging and prevention of diseases. The most potential antioxidant plants can be used in the formula of some supplement or herbal medicine. Myanma traditional medicine production has been using the medicinal plants which content the antioxidant activity. People are using the plenty of antioxidant food and medicines. This study work can also raise the role of the local traditional medicine with the scientifically experiment results by testing the antioxidant activity and support the traditional medicine production, pharmaceutical production and food and beverages production.

Acknowledgement

We would like to thank the Honourable Minister, Ministry of Science and Technology, Head of Department of Research and Innovation provided for this research, Department of Standards and Quality, National Analytical Laboratory and Analysis Department, U Aung Khine for identification of plant samples, Dr. Sint Soe (Rector) from Technological University (Mandalay), Dr. Hla Myat Mon (Visiting Professor) and colleagues.

References

Shekhar T.C and Anju. G.G.,'Antioxidant Activity by DPPH radical scavenging method of *Ageratum conyzoides* Linn. Leaves', American Journal of Ethnomedicine Vol(1), No.4, 2014, 244-249.

Zheng W, Wang S.Y: Antioxidant and Phenolic compounds in selected herbs, J. Agric. Food Chem., 2001. 49:5165-5170.

Pietta P.G. 'Flavonoid as antioxidants' J. Nat Prod. Jul; 2000, 63(7): 1035-42.

Jaegaer, A., Walti, M., and Neftel, K., 'Side effects of flavonoids in medical practice In plant Flavnoids in Biology and Medicine H: Biochemical, Cellular, and Medical Properties, Alan R. Liss. New York, NYUSA., 1988, p.373-374.

Cook N.C and Samman, S., 'Flavonoids-Chemistry, Metabolism, Cardioprotective Effects, and Dietary sources', Nutritional Biochemistry, 1996, 7:66-76.

V. Ani and Kamatham A. Naidu, 'Antioxidant Potential of Bitter Cumin (Centratherum anthelminticum) (L.) Kuntze. Seeds in in Vitro Model', BMC Complementory and Alternative Medicine, 2011, 11:40.

Evans, C. R., N Miller, N., Pagaga, G., 'Antioxidant Properties of Phenolic Compounds', Trands in Plant Science, 1997, 2(4): 152-159

Ministry of Agriculture, 'Burmese Medicinal Plants', Agricultural Corporation, (pa-sa-ka- No. 119708), 1980, p. 387-389.

Rastogi, R. P. and Mehrotra, B.N, 'Compendium of Indian Medicinal Plants and National Institute of Science Communication, Vol I, 1960-1969, New Delhi, 1990, pp. 33,78,136,137,192,200-203.

Rastogi, R. P. and Mehrotra, B.N, 'Compendium of Indian Medicinal Plants and National Institute of Science Communication, Vol II, 1970-1979, New Delhi, 1990, pp. 45-46, 143, 233, 234, 333, 334, 347-9.

Rastogi, R. P. and Mehrotra, B.N, 'Compendium of Indian Medicinal Plants and National Institute of Science Communication, Vol III, 1980-1984, New Delhi, 1990, pp. 41-42, 135-136, 220-221, 306-307, 319-320.

The Wealth of India, 'A Dictionary of Indian Raw Materials and Industrial Products, Vol II, India, Council of Scientific and Industrial Research, 1950, 77-82, 402-407.
13. Neha J., Mishra R.N., 'Antioxidant Activity of Trikatu mega Ext.' Int. Jour of Res in Phar and Biomed Sci. 2(2) :624-628, 2011.
International Organization for Standardization: ISO:1572:1980(E): 'Tea-Preparation of ground sample of known dry matter content', 2nd edition, UDC, 663, 95:620.113.
International Organization for Standardization: ISO:14502-1:2005(E): 'Determination of substances characteristic of green and black tea - Part 1: Content of total polyphenols in tea - Colorimetric method using Folin-Ciocalteu reagent', www.iso.org. published in Switzerland.
Chang, C. C., Yang, M. H., Yen, H. M., and Chern, J. C., 'Estimation of Total Flavonoid Content in Propolis by Two Complentary Colorimetric Methods'. Journals of Food and Drug Analysis, 10(3), 2002, 178-182.
Saboo, S.R., Tapadiya, R., Khadahadi, S.S., and Deokate, U.A., J. Chem. Pharm. Res., 2(3):417-423, 2010.
Moongkarndi, P., Kosem, N., Kaslungka, S., Luranratana O., Pongpan, N. and Neungton, N., Antiproliferation, antioxidation and inductio of apoptosis by Garcinia mangostana (mangosteen) on SKBR 3 human breast cancer cell line. J. Ethnopharmacol, 2004, 90, 161-6.
Ali Enas M., Phytochemical composition, antifungal, antioxidant, antiaflatoxigenic and anticancer activities of Glycyrrhiza glabra L. and Matricaria chamomilla L. essential oils, Journal of medicinal plants research, vol. 7 (29), 2013. pp. 2197-2207.
European Medicines Agency, Science Medicines Health, Assessment report on Glycyrrhiza glabra L. and/or Glycyrrhiza inflata Bat. and/or Glycyrrhiza uralensis Fisch., radix, Committee on Herbal Medicinal Products, An agency of the European Uinion, UK, p-16, www.ema.europa.eu., 2013.
Ramirez-Tortosa, M.C. et al., 1999, Oral administration of tumeric extract inhibits LDL oxidation and has hypocholesterolemic effects in rabbits with experimental altherosclerosis, 147:371-378, 2001.
Devasagayam, T. P. A., Kamat, J. P. and Sreejayan, N., Antioxidant action of Curcumin. In Micronutrients and Health: Molecular Biological Mechanisms. Nesaret nam K, Packer L (eds), AOCS Press, Champaign, IL, USA, p-42-59, 2001.
Devasagayam, T.P.A. and Sainis, K. B., Immune system and Antioxidant, especially those derived from Indian medicinal plants. Indian J. Exp. Biol., 2002, 40: 639-655.
Ammon, H.P.T and Wahl, M.A.. Pharmacology of Curcuma longa, Planta Med, 1991, 57:1-7.
Sharma, S.K., Goyal, N., In Vitro Antioxidant Activity of root extracts of Heliotropium eichwaldi Stued. ex DC, Research Journal of Pharmaceutical, Biological and Chemical Sciences, 3(1), 2012, 444-448.
Ayoola, G.A, Coker, H.A.B., Adesegun, S.A., Adepoju Bello, A.A., Obaweya, K., Ezennia, E.C., et al., , Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigera, Trop J. Pharm. Res., 2008, 7:1019-1024.
Chao, P.D.L, Hsiu, S.L., Hou, Y.C., Flavonoids in herbs: Biological fates and potential interactions with xenobiotics. J. Food Drug Anal, 2002, 10(4):219-228.
Hossain, M.A., Muhammad, M.D., Charles, G., Muhammad, M.D., Charles, G., Muhammad, I., In Vitro total phenolic, flavonoids contents and antioxidant activity of essential oil, various organic extracts from the leaves of tropical medicinal plant. Tetrastigma from Sabah. Asian Pac J. Trop. Med, 2011, 4(9): 717-721
Kokate, K.C., Practical Pharmacognosy, 4th ed. Delhi: Vallabh Prakashan, p. 218, 1997.

Qualitative Analysis of Tetracycline Residues and Evaluation of Antibiogram of *Salmonella* and *Vibrio* Isolates from Whiteleg Shrimp (*Litopenaeus Vannamei*)

TAY-ZAR AYE CHO, THAZIN OO, ANIL KUMAR ANAL

Faculty of Food Engineering and Bioprocess Technology, Asian Institute of Technology, Thailand

Abstract

Bacterial pathogens and their ability to resist certain antibiotics in shrimps poses a serious threat to food safety. Moreover, drug residues in the meat adversely effect the quality and safety of shrimp. In this study, whiteleg shrimp (*Litopenaeus vannamei*) were collected from ten retail shops from a local wet market. Each shop was visited twice, totaling 20 samples. *Salmonella* and *Vibrio* species were isolated from shrimp samples. Shrimps from 40% and 30% of retail shops were positive for *Salmonella* and *Vibrio,* respectively. Isolates were subjected disk diffusion method to test the antibiotic susceptibility against nine common antibiotics, namely Amoxicillin, Ampicillin, Ceftriaxone, Sulfamethoxazole, Chloramphenicol, Ciprofloxacin, Gentamycin, Streptomycin and Tetracycline. All the *Salmonella* isolates were multi-drug resistant, showing resistance to ampicillin, amoxicillin and tetracycline. Fifty percentage of those *Salmonella* isolates were further resistant to sulfamethoxazole. None of the *Vibrio* isolate was resistant to all the antibiotics tested. However, all *Vibrio* isolates were tested as intermediate resistant to sulfamethoxazole. Isolates that were resistant to any antibiotic tested were further analyzed for the presence of antibiotic resistance genes. Bacterial DNA were amplified for the gene coding tetracycline (*tet(A)* and *tet(B)*) and beta-lactams (bla_{SHV} and bla_{CMY}). Gel electrophoresis results showed 50% of the *Salmonella* isolates were positive for tetracycline resistant genes, *tet(A).* Genes coding for beta-lactams (bla_{SHV} and bla_{CMY}) were not detected in the isolates even though they were phenotypically resistant to ampicillin and amoxicillin. Following residue tests, shrimps from 50% of retail shops were detected as positive for tetracycline residue. The present study highlights the presence of *Salmonella* and *Vibrio* species in shrimp samples which could lead to foodborne disease outbreaks. Possible transfer of resistant genes to other bacteria also raises the growing concern for emergence of multi-drug resistant bacteria. Additionally, presence of antibiotic residues in shrimp may pose not only to safety but also to economic loss.

Introduction

It has been widely acknowledged that improper use of antibiotics in animal and food production poses a serious threat in the development of antibiotic resistant pathogens which can infect both animals and humans (Khachatourians, 1998; Willis, 2000). There are extensive reports on the emergence of antibiotic resistant pathogens in aquaculture environment and subsequent transfer of resistant genes to other environments have been well documented (Inglis, 2000; Rhodes et al., 2000; Sørum, 1999). Intensive farming of fish and shrimp has promoted the growth of several bacterial diseases, which has led to an increase in the use of antimicrobials (Defoirdt et al., 2011, 2007). Extensive usage of antibiotics in shrimp culture is responsible for the accumulation of residues in the aquatic environment and the emergence of antimicrobial resistant bacteria (Martinez, 2009).

Thailand is one of the top shrimp producers in the world and exports a huge amount to other countries yearly (FAO, 2005). According to the reports, *Salmonella* and

Vibrio species are major public health concern for seafood related food-borne diseases in Thailand (Angkititrakul et al., 2005; Minami et al., 2010; Padungtod et al., 2007; Padungtod and Kaneene; 2006). Among different species. whiteleg shrimp (*Litopenaeus vannamei*) is the most commonly consumed shrimp in Thailand and South East Asian countries. Nevertheless, data are lacking on the prevalence of pathogenic bacteria and antibiotic resistance profile of these bacteria in shrimps at retail level. Furthermore, presence of antibiotic residues in the meat should also be accessed.

The objectives of the current study are to detect the occurrence of *Salmonella* and *Vibrio* from whiteleg shirmps in wet market and to identify the antibiotic resistance profiles of these pathogens as well as the drug residues in shrimp meat.

Materials and Methods

Sampling collection

Overall, 20 samples of whiteleg shrimps were collected from ten retail shops in Talad Thai whole sale wet market, Pathumthani province, Thailand. Samples were transported in the ice-box and processed within the same day of collection in the Biotechnology Lab, Asian Institute of Technology.

Isolation and identification of *Salmonella* and *Vibiro*

The summary of isolation and identification procedures for *Salmonella* and *Vibrio* are outlined in Figure 1. Briefly, a 25g portion of each sample was homogenized with 225ml of sterilized alkaline peptone water and incubated at 37˚C for 24h. For isolation of *Salmonella*, 0.1ml from each pre-enriched sample was inoculated into 10ml of Rappaport-Vassiliadis broth (Himedia, India) and incubated at 37˚C for 24h. A loopful of inoculum from each sample was subsequently streaked onto XLD agar (Himedia, India). For *Vibrio* species, pre-enriched samples were streaked onto *Vibrio* selective TCBS agar (Himedia, India) and incubated at 37˚C for 24h. Up to five presumptive colonies from each plate were Gram stained and further subjected to biochemical testes, namely Triple Sugar Iron, Urease, IMViC (Indole, Methyl red, Voges-Proskauer & Citrate), Lysine Decarboxylase and Oxidase tests (Himedia, India). Confirmed isolates were inoculated into nutrient broth with 15% (v/v) glycerol and stored at -20˚C for further tests.

Antibiotic susceptibility test

Antibiotic resistance profile of *Salmonella* and *Vibrio* isolates were tested by disk diffusion method against nine common antibiotics; Amoxicillin (10µg), Ampicillin (10µg), Ceftriaxone (30µg), Sulfamethoxazole (25µg), Chloramphanicol (30µg), Ciprofloxacin (5µg), Gentamycin (10µg), Streptomycin (10µg) and Tetracycline (30µg), all from Oxoid (UK), following the Clinical and Laboratory Standard Institute (CLSI, 2013) guidelines. Bacterial suspensions in nutrient broths were adjusted to 0.5 McFarlane standard (10^8 CFU/ml) and incubated onto Mueller-hinton agar (Himedia, India). Antibiotic disks were placed onto the agar and incubated at 37˚C for 24h. Results were interpreted by measuring the diameter of inhibition zone in millimeter around the antibiotic disks. Following CLSI standards, antibiotic resistance of bacteria were categorized into susceptible, intermediate or resistant (CLSI, 2013).

Detection of antibiotic resistance genes

Isolates which showed fall into ‘intermediate’ and ‘resistant’ categories in disk diffusion methods were examined for the presence of antibiotic resistant genes

coding for beta-lactams (Amoxicillin and Ampicillin) and tetracycline. Genomic DNA were extracted from *Salmonella* and *Vibiro* isolates grown overnight on nutrient agar (Himedia, India) using Insta-max Gene Matrix (Bio-rad, Germany) following manufacturer's instruction. Primers designed for specific detection of antibiotic resistant genes, beta-lactams (*bla$_{SHV}$, bla$_{CMY}$)* and tetracycline (*tet(A), tet(B)*), were used (Table 1). PCR assay was performed in a total volume of 10 µl, including 0.5 µl of every 10 µM primers, 8 µl of PCR buffer, $MgCl_2$, *taq*DNA polymerase, water, 10 mM dNTP and 1µl of template DNA. Amplification reactions were carried out by using a DNA thermo-cycler (Eppendrof Mastercycler, Germany) as follows: 3min at 95°C, total 35 cycles each consisting of 1min at 94°C, 90s at different annealing temperatures and 1min at 72°C, followed by a final extension step of 10min at 72°C. Amplified PCR products were visualized by QIAxcel capillary gel electrophoresis system (Qiagen, USA).

Detection of antibiotic residues from shrimp

Shrimp meat samples were prepared in triplicate and tested for the presence of tetracycline residues using RR test kit designed for determination of drug residues in meat (Rodejanarug Pharmaceutical Limited Partnership, Thailand) following product's instruction. The kit detected the limits that are equal to the maximum residue limits for tetracycline group of antibiotics.

Figure 1: Outline of procedure for isolation and identification of *Salmonella* and *Vibrio* from shrimp samples collected from wet market.

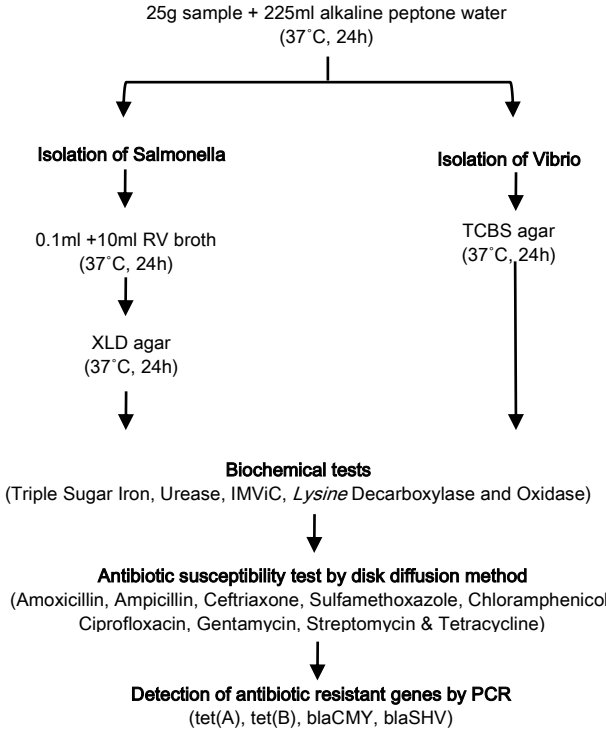


Table 1: Primers used to detect beta-lactams and tetracycline

Antibiotics	Genes	Nucleotide sequences (5-3)	Size (bps)	Tm (˚C)	Reference
Tetracycline	tet(A)	(F) GGTTCACTCGAACGACGTCA (R) CTGTCCGACAAGTTGCATGA	652	57	(Momtaz et al., 2012)
Tetracycline	tet(B)	(F) CCTCAGCTTCTCAACGCGTG R) GCACCTTGCTGATGACTCTT	634	56	(Momtaz et al., 2012)
Beta-lactams	blaSHV	(F) TCGCCTGTGTATTATCTCCC (R) CGCAGATAAATCACCACAATG	768	52	(Randall et al., 2004)
Beta-lactams	blaCMY	(F) TGGCCAGAACTGACAGGCAAA (R) TTTCTCCTGAACGTGGCTGGC	462	47	(Randall et al., 2004)

Results

Isolation and identification of *Salmonella* and *Vibrio*

In this study, 20 samples of whiteleg shrimps were collected from 10 retail shops in a Talad Thai wholesale wet market. The reason for choosing this location was that the Talad Thai market distributed fresh meat to the Bangkok and neighboring regions. Presence of pathogenic bacteria in shrimp samples from this market poses a high risk for a large urban population in Bangkok and neighboring districts. Shrimps sample were collected twice from each randomly assigned retail shop in the period of one week interval.

Salmonella and *Vibrio* were isolated from 40% and 30% of the retail shops, respectively (Table 2). Both *Salmonella* and *Vibrio* were isolated from one retail shop. In other words, 20% of samples were positive for *Salmonella* and 15% were positive for *Vibirio.*

Table 2: Occurrence of *Salmonella* and *Vibrio* in whiteleg shrimp from wet market

Sample	Bacteria	Retails collected (no.)	Retails with positive result (no.)	Occurrence (%)
Whiteleg shrimp	*Salmonella*	10	4	40.0
	Vibrio	10	3	30.0

Antibiotic susceptibility test

Antibiotic resistance profile of *Salmonella* and *Vibrio* isolates were presented in Table 3 and 4. All *Salmonella* isolates were found to be multi-drug resistant while all three *Vibrio* isolates showed intermediate resistance to Sulfamethoxazole only. All *Salmonella* isolates were resistant to amoxicillin, ampicillin and tetracycline while 50% of *Salmonella* were additionally resistant to sulfamethoxazole.

Table 3: Antibiotic resistance profile of *Salmonella* isolates from whiteleg shrimp samples

No.	Bacteria	Isolate no.	Resistance	Susceptibility
1	Salmonella	S1	AMC, AMP, TET	CRO, SXT, CMP, CIP, GEN, STR
2	Salmonella	S8	AMC, AMP, SXT, TET	CRO, CMP, CIP, GEN, STR
3	Salmonella	S10	AMC, AMP, SXT, TET	CRO, CMP, CIP, GEN, STR
4	Salmonella	S17	AMC, AMP, TET	CRO, SXT, CMP, CIP, GEN, STR

AMC – Amoxicillin, AMP – Ampicillin, CRO – ceftriaxone, STX – sulfamethoxazole, CMP – chloramphenicol, CIP – ciprofloxacin, GEN – gentamycin, STR – streptomycin, TET – tetracycline

Table 4: Resistance patterns of *Salmonella* and *Vibrio* isolates to different antibiotics

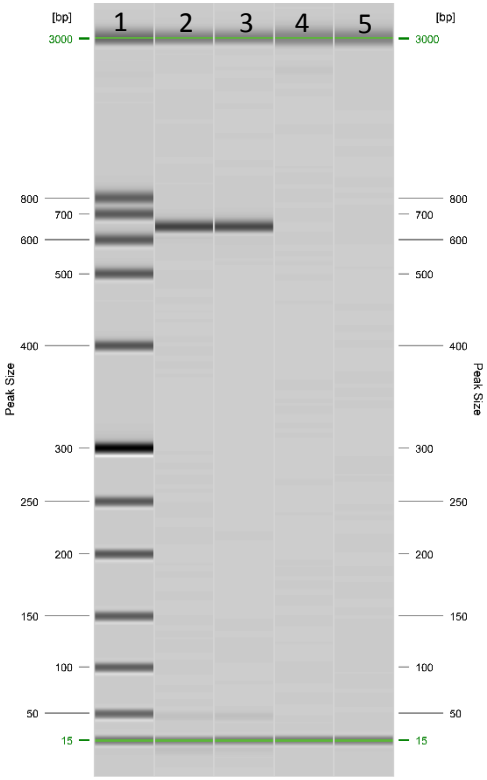
Antibiotics	*Salmonella* (n=4)	*Vibrio* (n=3)
Amoxicillin	4 (100%)	NR
Ampicillin	4 (100%)	NR
Ceftriaxone	NR	NR
Sulfamethoxazole	2 (50%)	NR
Chloramphenicol	NR	NR
Ciprofloxacin	NR	NR
Gentamycin	NR	NR
Streptomycin	NR	NR
Tetracycline	4 (100%)	NR
Resistance to 1 class of antibiotic	NR	NR
Resistance to 2 classes of antibiotic	NR	NR
Multi-drug resistance*	4 (100%)	NR

*Resistant to three or more classes of antibiotic, NR – No resistance

Detection of antibiotic resistant genes

Salmonella isolates that showed resistance in disk diffusion methods were further analyzed for the presence of genes coding for tetracycline and beta-lactams (Ampicillin and amoxicillin). Of four isolates, isolates S1 and S17 showed positive for *tet(A)* which were visualized at 650 bps in the gel image (Figure 2).

Figure 2: Gel image of *Salmonella* isolates from whiteleg shrimp samples. [Lane 1; Size marker (50-800 bps), Lane 2; Sample S1 with *tet(A)* genes positive; Lane 3; Sample S17 with *tet(A)* genes positive, Lane 4 and 5; Sample S10 and S8 without positive band.]



Detection of antibiotic residues from shrimp

Using RR test kit, tetracycline residues were detected in shrimps samples from 50% (n=5) of retail shops (Table 5). In those retail shops, residues were detected only once out of twice sample collections for each shop. Results were confirmed by producing triplicates for each sample.

Table 5: Screening of tetracycline residues in whiteleg shrimps from different retail shops

Sample	Antibiotic	Retails collected (no.)	Retails with positive result (no.)	Occurrence (%)
Whiteleg shrimp	Tetracycline	10	5	50.0

Discussion and conclusion

The occurrence of pathogenic bacteria in meat and meat products put a serious threat on food safety. In the presence study, *Salmonella* and *Vibrio* were isolated from 40% and 30% of the samples, respectively. Dalsgaard and coworkers (1995) reported that none of the 158 samples from shrimp production areas in Thailand were positive for *Salmonella* while 35% of 107 samples were positive for *Vibrio* species. Occurrence of pathogens in open markets favors the possible cross-contamination among food products. Sampling location selected in this study is a whole sale market that distributes foods around Bangkok and neighboring regions and hence the presence of *Salmonella* and *Vibrio* in retail shrimps may lead into foodborne disease outbreaks.

Increase in antibiotic resistant among bacteria in aquatic environment not only increase the chance of transfer of resistance genes to other bacteria but also adversely effect in the treatment of bacterial disease of fish and shrimps. In the present study, the occurrence of multi-drug resistant *Salmonella* in shrimps indicates the improper use of antimicrobial agents in aquaculture. In Thailand, *Salmonella* and *Vibrio* species derived from human sources are commonly resistant to a number of antibiotics such as chloramphenicol, sulfamethoxazole, trimethoprim and gentamycin (Boonmar et al., 1998; Dalsgarrd et al., 200). All the *Salmonella* isolates in this study were resistant to tetracycline, amoxicillin and ampicillin. This is the indication of mishandling of those drugs in shrimp industry in Thailand. Antibiotic resistance profiles of *Salmonella* in this study are similar to those from human studies recently reported in Thailand (Sanpong et al., 2010; Wannaprasat et al., 2011). Compare to the previous studies, *Vibrio* species in this study did not show resistant to all the antibiotics tested. Nevertheless, intermediate resistance to sulfamethoxazole has been found in all the *Vibrio* isolates which might show the possible threat of sulfamethoxazole resistant *Vibrio* species in the aquatic environment.

In *Salmonella,* most tetracycline resistant genes are encoded by *tet* genes (Butaye et al., 2003). In the present study, only one isolate from those which were seen as resistant in disk diffusion method was detected as positive for *tet(A)* gene. The primers used in the current study did not cover the whole range of antibiotic resistant genes for the selected antibiotics. This might be the reason that phenotypically resistant bacteria were not detected by PCR in this study. Those which were not detected by PCR might possess the resistance genes for the same antibiotics which are different from the targeted genes used in this study.

Presence of antibiotic residues in meat samples has long been discussed widely. Due to the limitation of test kit, only tetracycline has been tested in this study. The presence of tetracycline residue in 50% of retail shops poses a high risk of public health concern. Very few data are available in the literature regarding the presence of antibiotic residues in shrimps in Thailand. Antibiotic residues were found 21% of meat samples in Ghana (Donkor et al., 2011) and 44% in Nigeria (Stolker and Brinkman, 2005). Likewise, drug residues were found in 50% of meat samples in Iraq (Tajick and shohreh, 2006) and 70% in Tanzania (Kurwijila et al., 2006) . Interestingly, Goulette (2007) and Mmbando (2004) reported that higher proportion of tetracycline in animal source food compare to other antibiotic residues.

A large number of antibiotics are using in the shrimp farming in Thailand. Those drugs are used not only for therapeutic but also for growth promotion. Presence of *Salmonella* and *Vibrio* species in whiteleg shrimps from wet markets highlight the possible outbreak of foodborne disease. Furthermore, both pathogens and antibiotic residues in shrimp can lead to serious economic loss if export is not allowed due to food safety concerns.

This study also suggested the possibility of bacteria possessing different resistant genes for the same drug. A wide range of antibiotic resistant genes should be included in the future studies.

References

Angkititrakul, S., Chomvarin, C., Chaita, T. and Kanistanon, K. 2005. Epidemiology of antimicrobial resistance in Salmonella isolated from pork, chicken meat and human in Thailand. Southeast Asian. J. Trop. Med. Public Health., 36(6) : 1510 - 1515.

Boonmar, S., Bangtrakulnonth, A., Pornruangwong, S., Samosornsuk, S., Kaneko, K. & Ogawa, M. 1998. Significant increase in antibiotic resistance of Salmonella isolates from human beings and chickens in Thailand. Veterinary Microbiology, 62, 73–80.

Butaye, Patrick, Luc A. Devriese, and Freddy Haesebrouck. 2003. Antimicrobial growth promoters used in animal feed: effects of less well known antibiotics on gram-positive bacteria. Clinical microbiology reviews 16, no. 2: 175-188.

Dalsgaard, A., Forslund, A., Serichantalergs, O. & Sandvang, D. 2000. Distribution and content of class 1 integrons in different Vibrio cholerae O-Serotype strains isolated in Thailand. Antimicrobial Agents and Chemotherapy, 44, 1315–1321.

Dalsgaard, A., Hans Henrik Huss, A. H-Kittikun, and J. L. Larsen. 1995. "Prevalence of Vibrio cholerae and Salmonella in a major shrimp production area in Thailand." International journal of food microbiology 28, no. 1 :101-113.

Defoirdt, Tom et al. 2007. Alternatives to antibiotics to control bacterial infections: luminescent vibriosis in aquaculture as an example."In: Trends in biotechnology. 25 (10), pp. 472-9.

Defoirdt, Tom; Sorgeloos, Patrick; Bossier, Peter. 2011. Alternatives to antibiotics for the control of bacterial disease in aquaculture.". In: Current opinion in microbiology. 14 (3), pp. 251-8.

Donkor, E.S, Newman, M.J, Tay, S.C.K, Dayie, N, T., Bannerman, E. and Olu-Taiwo, M. 2011. Investigation into the risk of exposure to antibiotic residues contaminating meat and egg Ghana. Food Control., 22 : 869 – 873.

Food and Agriculture Organization. 2005. Aquaculture production, 2003. Year book of Fishery Statistics - Vol.96/2. Food and Agriculture organization of the United Nations, Rome, Italy.

Goulette, R.R. 2007. Investigation of Safe-Level Testing for Beta-lactam, Sulfonamide, and Tetracycline Residues in Commingled Bovine Milk. Pell Scholars and Senior Theses, Salve Regina University.

Inglis, V. 2000. Antibacterial chemotherapy in aquaculture: review of practice, associated risks and need for action. In: Use of Chemicals in Aquaculture in Asia (edited by J. R. Arthur, C. R. Larilla-Pitogo&R. P. Subasinghe), Pp. 7– 2. Proceedings of the Meeting on the Use of Chemicals in Aquaculture in Asia, 20–22 May 1996, Tigbauan, Iloilo, Philippines. Tigbauan, Iloilo, Philippines: Southeast Asian Fisheries Development Center Aquaculture Department.

Khachatourians, G.G. 1998. Agricultural use of antibiotics and the evolution and transfer of antibiotic-resistant bacteria. Canadian Medical Association Journal, 159, 1129–1136.

Kurwijila, L.R., Omore, A., Staal, S. and Mdoe, N.S.Y., 2006. Investigation of the risk of exposure to antimicrobial residues present in marketed milk in Tanzania. Journal of Food Protection, 69(10), pp.2487-2492.

Martinez, J. L. 2009. Environmental pollution by antibiotics and by antibiotic resistance determinants. Environmental Pollution 2009, 157, 2893–2902.

Minami, A., Chaicumpa, W., Chongsa-Nguan, M., Samosornsuk, S., Monden, S., Takeshi, K., Makino, S. and Kawamoto, K. 2010. Prevalence of food borne pathogens in open markets and supermarkets in Thailand. Food Control., 21: 221 – 226.

Mmbando, L.M.G. 2004. Investigation of oxytetracycline use and abuse: Determination of its residue in meat consumed in Dodoma and Morogoro. A thesis submitted for the award of a MVM Degree at Sokoine University of Agriculture, Morogoro, Tanzania, pp. 240.

Padungtod, P. andKaneene, J. B. 2006. Salmonella in food animals and humans in northern Thailand. Int. J. of Food Microbiology., 108(3):346-354.

Padungtod, P., Kadohira, M. and Hill, G. 2008. Food safety concepts and foodborne bacteria in food animals in Thailand, Laopdr and Vietnam. Chiangmai veterinary Journal., 5(2) : 113 - 121.

Rhodes, G., Huys, G., Swings, J. et al. 2000. Distribution of oxytetracycline resistance plasmids between Aeromonads in hospital and aquaculture environments: Implications of Tn1721 in dissemination of the tetracycline resistance determinant Tet A. Applied Environmental Microbiology, 66, 3883–3890.
Sapkota, A.; Sapkota, A. R.; Kucharski, M.; Burke, J.; McKenzie, S.; Walker, P.; Lawrence, R. 2008. Aquaculture practices and potential human health risks: Current knowledge and future priorities. Environ. Int. 2008, 34, 1215–1226.
Sørum, H. 1999. Antibiotic resistance in Aquaculture. Acta Veterinaria Scandinavia, Supplementum, 92, 29–36.
Stolker, A.A.M. and Brinkman, U.A.Th. 2005. Analytical strategies for residue analysis of veterinary drugs and growth-promoting agents in food-producing animals-a review. Journal of Chromatography., 1067: 15–53.
Tajick, M.A. and Shohreh, B. 2006. Detection of Antibiotics Residue in Chicken Meat Using TLC. International Journal of Poultry Science 5 (7): 611-612.
Wannaprasata, W., Padungtodb, P. and Chuanchuena, R. 2011. Class 1 integrons and virulence genes in Salmonellaenterica isolates from pork and humans. International Journal of Antimicrobial Agents, 37: 457-461.
Willis, C. 2000. Antibiotics in the food chain: their impact on the consumer. Reviews in Medical Microbiology, 11, 153–160.

Acute Toxicity ff Bisphenol A Induced Phenotypic Changes on Zebrafish (*Danio Rerio*) During Early Development

MAI NGO THI[1], NGOC VO THI KIM[2],
CHI DO HONG CHI[3], NGA LE PHI[4]

[1]Department of Occupational Hygiene and Health, Sub- Institute of Labour and Environment Protection in the South of Vietnam, Vietnam
[2] Ho Chi Minh City University of Science, Vietnam National University Ho Chi Minh City, Vietnam
[3] Vietnam National University Ho Chi Minh City, Vietnam
[4] Ho Chi Minh City University of Technology, Vietnam National University Ho Chi Minh City, Vietnam

Abstract

Bisphenol A (BPA) exhibits hormone-like properties that raise concern about its negative effects to human health through BPA releasing from consumer products, food containers and production of polycarbonate plastics and epoxy resins. Although initially consideration BPA is a weak environmental estrogen, more recent studies have demonstrated that this compound may be similar in potency to estradiol in stimulating some cellular responses and phenotype changes. In addition, the concern on the ecological impact of BPA to aquatic organisms has been increasingly raised on aquatic organisms at environmental relevant concentrations that potential human health effects of early – life exposure to BPA. This study used zebrafish *(Danio rerio)*, an aquatic animal, as a model for an toxicological testing of bisphenol A. The semistatic test procedure was followed to investigate the effects of BPA at concentrations of 5 mg/l, 6 mg/l, 7 mg/l, 8 mg/l and 9mg/l) to capture some endpoints during the early development as morphology effects, the lethal concentration 50 (LC_{50}) at 24h, 48h, 72h and 97h BPA exposure on 3 days on old zebrafish larvae. As the results, the $LC_{50\text{-}24hrs}$ = 9.503 mg/l, $LC_{50\text{-}48hrs}$ = 8.688 mg/l, $LC_{50\text{-}72hrs}$ = 7.328 mg/l and $LC_{50\text{-}72hrs}$ = 6.669 mg/l, were determined for BPA on 3 days old larvae. Phenotypic analysis using zebrafish larvae revealed BPA early – life exposure toxicity caused cardiac edema, cranio-facial abnormality. The result from this research provided relevant information for environmental and human risk assessments.

Keywords: Danio rerio, zebrafish, bisphenol A, acute toxicity.

Introduction

Bisphenol A (BPA) is one of the highest volume chemicals produced worldwide. This compound is a building block of polycarbonate plastics often used for food and beverage storage, and BPA is also a component of epoxy resins that are used to line food and beverage containers. Studies have shown that BPA can leach from these and other products in contact with food and drink, and as a result, routine ingestion of BPA is 0. An area of increasing interest in the potential of BPA exposure to contribute to obesity and its associated metabolic complications 0. Some epidemiological studies have associated diseases and a number of cardiovascular risk factors such as hypertension, obesity, diabetes, metabolic syndrome and atherosclerosis 0, 0, 0. BPA also is the one of the most abundant endocrine disruptors in the environment. Although initially considered to be a weak environmental estrogen, more recent studies have demonstrated that

BPA may be similar in potency to estradiol in stimulating some cellular responses. Moreover, emerging evidence suggests that BPA may influence multiple endocrine-related pathways.
Zebrafish (*Danio rerio*), a model organism of vertebrate development and organogenesis, is receiving increasing attention as a model for human diseases, drug discovery and toxicology studies. Presently, zebrafish embryo and larvae is included as one of the principal test organism within the Organization for Economic Cooperation and Development test guidelines for endocrine disrupting compounds (ECDs) 0, thus zebrafish is a relevant model for investigating BPA toxicity. The availability of zebrafish in the large numbers, its small size and easy husbandry makes the zebrafish a more cost effective model than the other models for toxicological studies, for instance, rodent or rat. Owning to the conserved developmental program within vertebrates, fish and mammals share many similar developmental processes. Many of the genes or molecules with essential functions found in human such as those involved in developmental processes and toxicological responses are also found in zebrafish 0.
The larval stages of fish are sensitive to environmental stressors and the use of fish larvae provides advantages for the testing and understanding of the toxic mechanism and environmental impacts of chemicals. As mentioned aboved, several researchers have focused on the toxicity of bisphenol A, however much less intention has been paid to the toxicity of BPA on the malformation. In the present study, BPA was chosen as examples to examine it individual effects on the *Danio rerio* larvae for 4 days from 3 day fertilized post (dfp) to investigate morphology changes during early – life development that was little know in the past. This larvae 4-day semistatic renew test, adopted from short term methods for aslo estimating chronic toxicity in freshwater organism during development.

Materials and methods

Materials

Chemical tested: Bisphenol A (BPA) (CAS no. 80-05-7) was purchased from Sigma Aldrich Co. (USA) and stock solution of 100mg/ml was prepared by dissolving the chemical in 0.025% ethanol (Merk, Germany).
Zebrafish: Wild- type adult zebrafish *(Danio rerio)* were initially purchased from a commercial source (Thu Duc District, Ho Chi Minh City, Vietnam). Female and male zebrafish were cultured in a glass aquarium aerated with a dissolved oxygen content approximate 90% of the saturated levels. The pH value was kept at 7.23±0.19. The water temperature was 26,0±0,19^{o}C with a light–dark period of 14:10 h. The fish were fed twice a day with dry flakes (Tetra TertraMin, >48% protein, UK). To ensure optimal water quality, any remaining food was removed daily. Embryos and larvae were obtained by natural mating with a male: female ratio of 2:1 and raised in embryo water (Hank's Buffered Salt Solution (HBSS)) 0 Spawning and fertilization took place within 30 min when the light was switched on in the morning. The embryos were then collected from the aquarium. A single mature female lays 100–300 eggs per day. The embryos were observed with a dissecting microscope to select the fertilized eggs for the experiments. The embryos were then rinsed with filtered water several times before being exposed to different concentrations of chemical agents.

Determination of LC_{50} of bisphenol A for zebrafish (*Danio rerio*) larvae

Developing zebrafish were exposed to nominal concentration of 5 mg/l, 6 mg/l, 7 mg/l, 8 mg/l and 9 mg/l BPA with final concentration 0,025% (v/v) ethanol (vehicle) in egg water for 96h (4 days) from 3 dfp (day fertilization post) onwards. Control fish were maintained in embryo water without vehicle and media was renewed daily (24h) because of reported half – life of bisphenol A changes between 2.5 and 4.0 days 0 Each solvent control and exposure concentration group used n=10 fishes with a total of six replicates. No food was given during this period, because the larvae were provided with nutrients from the attached yolk sac. Enpoints used for assessing developmental toxicity included mortality and malformation rate (cadiac edema and reduced blood flow) after 24h, 48h, 72h and 96h exposure. The parallels were set up to calculate the EC_{50} and LC_{50} values determined by EPA analysis program (Version 1.5) using Probit method (Tidepool Scientific Software, USA).

Statistical analyses

The LC_{50} values for the larvae, live and dead larvae were counted until experiments ended.

The difference among data was analyzed using one way analysis of variance. The results are expressed as the mean ± standard error of the mean and values of $P< 0.05$ was considered to be statistically significant.

Results and discussion

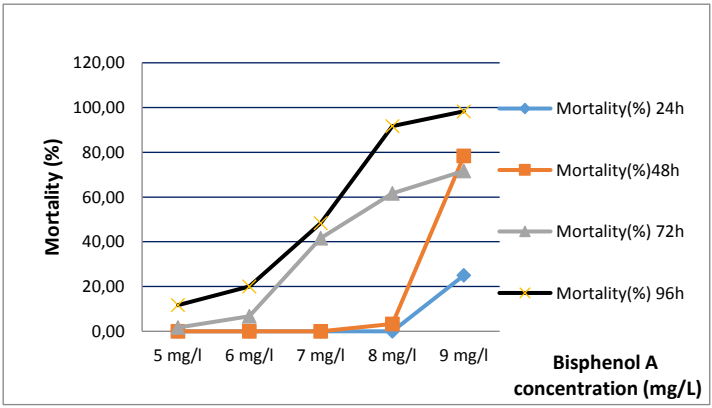
The LC_{50} values of BPA for 96h (4 days) from 3 dpf (day post - fertilization) *Danio rerio* larvae

This study was conducted to determine the acute toxic effects of BPA on *Danio rerio* larvae form 3 dpf (day post - fertilization) for 4 days. The LC_{50} values calculated from the *Danio rerio* larvae and 95% confidence limits are shown in table 1. The 24h, 48h, 72h and 96h LC_{50} value for the *Danio rerio* larvae was determined as 9.503 mg/l (9.184-10.565), 8.688 mg/l (8.560-8.881), 7.681 mg/l (7.414-7.989) and 6.669 mg/L (5.654 - 7.607), respectively (Table 1). The mortality rate (%) increased with an increase in the BPA concentration. At the end of 96h, all of the larvae exposed to 9 mg/L of BPA were dead (Fig.1).

Table 1: LC50 values and corresponding confidence intervals for zebrafish larvae exposed to BPA at 24, 48, 72 or 96h.

Time (hrs)	LC_{50}/EC_{50} (mg/l)	95% CL (lower/upper)
24h	9.503	9.184 -10.565
48h	8.688	8.560 - 8.811
72h	7.681	7.414 -7 .989
96h	6.669	5.654 - 7.607

Figure 1: 24h – 48h – 72h – 96h LC50/EC50 value of bisphenol A for exposed zebrafish larvae at 5 mg/l, 6mg/l, 7mg/l, 8 mg/l and 9 mg/l concentrations



The acute toxicity of BPA on aquatic organism has been studied using several species in the past decades. Acute studies on some fish species showed performed là 96h LC_{50} values là 4.7, 3 - 4, and 7.5 mg/L for *Fathead minnow*, *Oncorhynchus mykiss*, and *Cyprinodon variegates*, respectively 0. Alexander et al 0 studied the acute effects of BPA and reported a 96h LC_{50} value of 4.7 mg/L (4.0 - 5.5) using the static test method and 4.6 mg/L (3.6 - 5.4) using the test method for semistatic for *Pimephales promelas.* Yokota et al 0. The studied effects of BPA on the early life stages in Japanese Medaka (*Oryzias latipes*) and 96 h LC_{50} value observed of 13.0 mg/L (11.6 - 14.7). The lower 96 h LC_{50} value observed in the study for *Chalcalburnus tarichi* larvae derived from an interspecies BPA against susceptibility by Ertuğrul Kankaya et al 0.

The acute toxicity of bisphenol A was conducted in variation of other testing models, for examples: zebrafish liver cell line (ZFL) 0, *Daphania magna* 0. Although *Danio rerio* embryo and larvae were considerate an suitable models with advantages for testing chemicals, especially endocrine disrupting compounds (EDCs) 0, there were not many reports using them for testing BPA acute toxicity 00. Chow et al 0 studied the acute effects of BPA for *Danio rerio* and reported a 96h LC_{50} value of 8.041 mg/L (7.846 - 8.24). The 96h LC_{50} value of BPA for *Danio rerio* larvae in this study (6.669 mg/L (5.654 - 7.607)) also was lower with those previously reported. On the other hands, according to current EPA standard evaluation procedures, BPA was moderate to slightly toxic to fish and invertebrates with LC_{50} or EC_{50} values of 1.1 to 10 mg/l 0. Thus the test systems used in this study appeared to be correct for evaluation the toxicity of BPA.

Phenotype analysis of BPA early life exposure toxicity in *Danio rerio* larvae

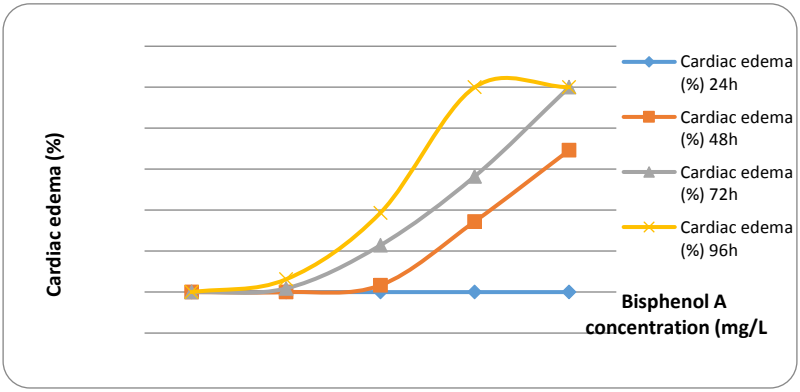
In this study, cardiac edema (CE) was not observed at 5 mg/l BPA concentration from 24h-96h. This malformation was observed at 48h beginning with 7mg/l, 8 mg/l and 9 mg/l concentrations with 3.33%, 34.48% and 69.23%, respectively, and there was not observed at 6 mg/l BPA concentration. At 72h, cardiac edema was observed at 6 mg/l BPA concentration (1.79%), and the percentage of

cardiac edema was increased at with 7mg/l, 8 mg/l and 9 mg/l concentrations with 22.86%, 56.52% and 100%, respectively. Deformities level observed were increased with the increase of BPA concentration and exposure time. At 96h, all of the surviving zebrafish larvae exposed to 8 mg/l and 9 mg/l BPA was cardiac edema (Fig.2, Table 2).

Table 2

	BPA concentration (mg/l)				
CE (%)	5	6	7	8	9
24h	0.00	0.00	0.00	0.00	0.00
48h	0.00	0.00	3.33	34.48	69.23
72h	0.00	1.79	22.86	56.52	100.00
96h	0.00	6.25	38.71	100.00	100.00

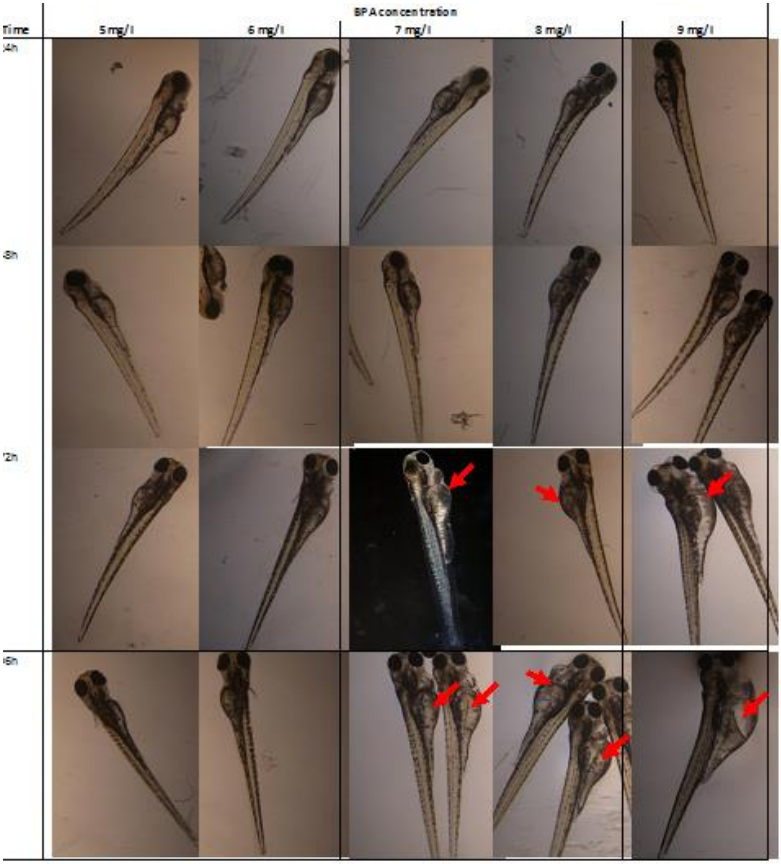
Figure 2: Cardiac edema (CE) (%) of zebrafish larvae at different concentrations of bisphenol A after 24h, 48h, 72h and 96h exposure. Percentage of cardiac edema is the ratio of the number of cardiac edema zebrafish larvae and the total of zebrafish larvae survived at 24h, 48h, 72h and 96h exposure.



Cardiac edema is defined that is a manifestation of congestive heart failure, due to increased venous and capillary pressures and often associated with renal sodium retention. There was little evidence of the abnormality of morphology in zebrafish exposed to BPA with high concentration in the short term that was reported in the past. Ertuğrul Kankaya et al. 0observed cardiac edema in *Chalcalburnus tarichi* larvae at BPA concentration 0.75, 1.5 and 3 mg/l included with slowed heart rate and blood circulation, developmental arrest, yolk sac edema, spinal deformity, tail abnormalities, regression in the pigmentation, regressed swim bladder formation and delayed yolk sac withdrawal when compare to controls. Duan et al 0 observed cardiac edema on zebrafish embryo at 72h 20.87 mg/l. Lam SH et al 0 studied the acute effects of BPA reported cardiac edema was observed in developing zebrafish larvae treated with 1.5 mg/L and 4.5 mg/l BPA for 7 days form 3 hours post fertilization (hpf) when compared to control. In addition, fish with cardiac edema also appeared to have cranio-facial abnormality (broad – headed (branchycephalic) and lacks anterior lower jaw protrusion), problem with swim bladder development/inflation and apparent gastro – intestinal abnormalities and partial yolk sac re- absorption. In this study, the

overall findings indicate that BPA caused dose-dependent adverse effects as a result of early – life exposure toxicity in zebrafish larva. In this study, cardiac edema (CE) was not observed at 5 mg/l BPA concentration from 24h-96h. The concentration exposure was induced cardiac edema (CE) was begun at 6mg/l BPA concentration at 72h, at 7 mg/l, 8 mg/l and 9mg/l BPA concentration at 48h. Cardiac edema induced BPA concentration was higher than Lam SH et al previously reported 0. Because of the difference of day post - fertilization beginning BPA exposure to zebrafish larvae, we must be more investigating for assessment of adverse effects of bisphenol A on the development and physiology of zebrafish during early – life.

Figure 3: Visualization of observed normal and abnormal zebrafish larvae exposed to BPA from 3 day post fertilization post. The results from microscopic examinations of larvae were categorized according to the concentration, time exposure and severity. Arrows were the cardiac edema positions on zebrafish larvae.



Conclusions

Consequently, this present study provided more insights in into BPA early – life exposure toxicity in the zebrafish larvae involving malformation during early life, especially cardiac edema. The results confirm that bisphenol A was toxic to the development of zebrafish larvae.

Conflict of interest

The authors declare no conflict of interests.

Acknowledgements

This work was funded by the Program of Young Technological Innovative Garden, Ho Chi Minh Scientific and Technological Department. The author wish to thank everyone who contributes to this study, especially the Department of Toxicity Lab, Insitute for Environment and Resources, Vietnam National University, Ho Chi Minh City for their editorial assistance. The using zebrafish larva in this study was conducted by accordance with national and institutional guidelines for the protection of human subjects and animal welfare.

References

Alexander, H.C., Dill, D.C., Smith, L.W., Guiney, P.D. and Dorn, P. " Bisphenol a: Acute aquatic toxicity." Environmental Toxicology and Chemistry 7 (1988): 19–26.

Chen MY1, Ike M, Fujita M. "Acute toxicity, mutagenicity, and estrogenicity of bisphenol-A and other bisphenols." Environ Toxicol 17, no. 1 (2002): 80-6.

Chow WS, Chan WK, Chan KM. "Toxicity assessment and vitellogenin expression in zebrafish (Danio rerio) embryos and larvae acutely exposed to bisphenol A, endosulfan, heptachlor, methoxychlor and tetrabromobisphenol A." J Appl Toxicol, 2013: 670-8.

Duan Z, Zhu L, Zhu L, Kun Y, Zhu X. "Individual and joint toxic effects of pentachlorophenol and bisphenol A on the development of zebrafish (Danio rerio) embryo." Ecotoxicol Environ Saf 71, no. 3 (2008): 774-80.

Ertuğrul Kankaya, Burak Kaptaner, Abdulahad Doğan ,İsmail Çelik. " Toxicity of bisphenol a during the early life stages of Chalcalburnus tarichi (Pallas, 1811)." Fresenius Environmental Bulletin 24, no. 3a (2015): 977-985.

Lam SH, Hlaing MM, Zhang X, Yan C, Duan Z, Zhu L, et al. "Toxicogenomic and Phenotypic Analyses of Bisphenol-A Early-Life Exposure Toxicity in Zebrafish." PLoS ONE 6, no. 12 (2011): e28273.

Lang IA, Galloway TS, Scarlett A, Henley WE, Depledge M, Wallace RB, Melzer D. "Association of urinary bisphenolA concentration with medical disorders and laboratory abnormalities in adults." JAMA 300, no. 11 (2008): 1303-10.

Lind PM1, Lind L. "Circulating levels of bisphenol A and phthalates are related to carotid atherosclerosis in the elderly." Atherosclerosis 218, no. 1 (2011): 207-13.

Melzer D1, Rice NE, Lewis C, Henley WE, Galloway TS. "Association of urinary bisphenol a concentration with heart disease: evidence from NHANES 2003/06 ." PLoS One 5, no. 1 (2010): e8673.

In No 47: Detailed review paper on fish screening assays for the detection of endocrine active substances, by OECD. ENV/JM/MONO18, 2004.

OECD. "Test No. 234: Fish Sexual Development Test ." 2011.

RR, Newbold. "Prenatal exposure to diethylstilbestrol (DES)." Fertil.Steril 89 (2008): e55-6.

Staples, C.A., Dorn, P.B., Klecka, G.M., O'block, S.T. and Harris, L.R. "A review of the environmental fate, effects, and exposures of bisphenol-A." Chemosphere 36 (1998): 2149–2173.

Vandenberg LN1, Hauser R, Marcus M, Olea N, Welshons WV. "Human exposure to bisphenol A (BPA)." Reprod. Toxicol 24, no. 2 (2007): 139-77.
Yang J, Chan KM. " Evaluation of the toxic effects of brominated compounds (BDE-47, 99, 209, TBBPA) and bisphenol A (BPA) using a zebrafish liver cell line, ZFL:." Aquat Toxicol, 2015: 138-47.
Yokota, H., Tsuruda, Y., Maeda, M., Oshima, Y., Tadokoro, H., Nakazono, A., Honjo, T. and Kobayashi, K. "Effect of bisphenol A on the early life stage in Japanese medaka (Oryzias latipes)." Environmental Toxicology and Chemistry 19 (2000): 1925–1930.

Assessing Workers Health Risk due to Fugitive Emission during Chemical Process Design Phase

MIMI H. HASSIM

Universiti Teknologi Malaysia, Department of Chemical Engineering, Malaysia
Aalto University, School of Chemical Technology, Finland

Paper Abstract

EU directives, such as the IPPC requires inclusion of safety, environmental and health analyses in process design as part of strategies towards achieving sustainable process development. In petrochemical and organic chemical industries which handle mostly volatile compounds, the most significant contributor to atmospheric releases is fugitive emissions from piping fittings and components. Fugitive emissions are not only a concern to economy and environment, but also a major source of background exposure to workers that may lead to various diseases including cancer. Each year more people die from occupational diseases than being killed in industrial accidents. And as for chemical process industries, the biggest threat to workers' health comes from fugitive emissions – the silent, unnoticeable, continuous releases mostly contributed by process piping and fittings.

In order to assess the potential health risk to workers in chemical plants, it is imperative to first, estimate the occupational air concentration due to fugitive emissions. For such an important aspect of protecting workers' welfare, the earlier the estimation, the better – the best is to conduct such assessment starting from the new process development and design phase.Operating plants regularly measure release and concentration levels through a plant-monitoring program. However for processes which are still 'on paper', predictive estimation methods are needed. Therefore in this work, newuser-friendly methods for quantifying fugitive emission rate and occupational air concentration are presented. Three methods are proposed for early design stages based on data available from simple piping flow diagrams (PFDs), detailed PFDs or piping and instrumentation diagrams (PIDs). The method becomes more comprehensive as it progresses from simple PFDs to PIDs since more process data is available later including plot plan, coordinates of the emission sources and local wind speed. Users however, can choose which method to use depending on the process information available in hand. The methods are demonstrated on a real industrial case study of benzene production in Borealis Polymers Oy plant at Porvoo, Finland. Subsequently, the estimated chemicals concentration values are used as input data for calculating the associated health risk.

Keywords: occupational health, fugitive emissions, occupational air concentration, process development, process design

PART II: Papers on Information and Communication Technologies……...

Smart Cities and the Elderly: Tensions Between Inclusion and Efficiency

TATJANA TODOROVIC, ARUL CHIB
Nanyang Technological University, Singapore

Abstract

Smart City concept emerges as a great opportunity to address a number of issues related to a worldwideageing population trends, such assteady economic development and increased wellbeing of its citizens, and a number of concurring ageing issues–successful and healthy ageing and social inclusion of senior citizens. The solution is found in smart technologies, promising efficiency and productivity improvements. Hollands alerts about the underlying emphasis on economic growth predicament, with the biggest challenge posed for Smart City managers to "effectively balance the needs of the community, with both those of local government and the needs of business." (2008, 309)

Thus, this study aims to firstly explore conceptual issues related to smart cities,in particular in relation to elderly residents, which may affect future elderly engagement with smart technologies. At the heart of Smart City concept stand two contradictory objectives – the stress on efficiency and productivity on one side, and wellbeing on another.Senior citizens' participation in a system they do not belong to or see fit to joinin seems highly unlikely. As a result, Smart City it is more likely to create new or deepen existing divides among seniors as the literature on elderly adoption of technologies indicates. Secondly, we hope to contribute to resolution of Smart City concept contradiction and explore possibilities for the further implementation strategies. Main objectiveis to provide constructive insights and general guidelines for policymakers towards more inclusive development and successful implementation of smart city related policies. Most of all, we hope to show that the careful consideration of elderly-sensitive contexts and needs to an inclusion of elderly in crafting technologies and policies will ensure more successful transition and truly inclusive Smart City.

Keywords: Smart City, elderly, digital divide, inclusion, technology adoption

Smart Cities and the Elderly: Tensions Between Inclusion and Efficiency

Emergent phenomenon of Smart Cities: definition andopportunities

Smart City concept emerges as a part of current discourses among policy makers, urban planners, technological development and other related sectors, as a great opportunity to address a number of issues related to sustainability and worldwide ageing population trends. With efficiency and productivity improvements, Smart City is promoted and developed by both private and public sector as an answer to a number of concurring issues –steady economic development, increased wellbeing of its citizens, successful and healthy ageing, even digital divide and urban

inequalities. The solution is found in the employment of the newest technologies, starting from computers, Internet and mobile phones, to the more recent smart technologies and features such as sensors, Internet of Things (IoT), telemedicine and service robots, among many others.While the precise definition of Smart City is still uncleargeneral understanding is that smart city heavily relies on utilization of smart technologies and big data for the more efficient development and functioning of cities (Yin et al. 2015; Hollands 2008; Caragliu et al. 2011).Hollands gives more specific definition in which smart city stands for "utilization of networked infrastructure to improve economic and political efficiency and enable social, cultural, and urban development" (2008, 308). Another comes from Yin et al: "a smart city is a system integration of technological infrastructure that relies on advanced data processing with the goals of making city governance more efficient, citizens happier, businesses more prosperous and the environment more sustainable." (2015, 6)

The point to note, in an attempt to define smart city potential, on one side is the emphasis on technologies that would sustain sustainable economic development and growth, which goes hand-in-hand with efficiency and productivity, cost savings, resource allocation and management, among many others. Private sector such as Frost and Sullivan, a growth consulting multinational company, naturally sees Smart City as a great market opportunity, especially for multi-business companies, stressing on efficiency and effectiveness of running urban networks, such as energy, infrastructure or healthcare services management, among others (press release Nov 7, 2014).On the other side of Smart Cities discourse, apart from economic growth and progress, is the "humanist' emphasis" as Hollands describes it (2008, 309), a notion that smart technologies could greatly improve living conditions and the wellbeing of its citizens(Caragliu et al. 2011, 68; Piro et al. 2013, 169; Bianchini and Avila 2014, 36).

The technology development sector is in particular quick to promise improved wellbeing based on the technological capabilities. As such, joint report on Smart Mobile Cities by Accenture, Cisco and the GSMA, leading companies in technology consultancy, networking and mobile operators, state that utilization of smart mobile technologies and data analysis will in due course ensure, among other things, "increasing GDP growth" and "the creation of a sustainable environment and the enhanced wellbeing of the citizens" (Accenture, Cisco, Inc and GSMA 2011, 6).Furthermore, connectivity, access to information and exchange, are seen as potential drivers to solve issues such as social and digital inequalities (Phipps 2000). As such, the goal of Korea's U-City (shorter for Ubiquitous City, one 'living' example of Smart City) is to ensure universal access, for more convenient, secure, inclusive information society, and moreover "humane way of life"(Shin 2009, 516). Connectivity that technologies allow is frequently included as the most contributing factor that would empower its users and therefore increase the quality of life (Komninos et al.2013, 120).Investments in human and social capital will contribute towards participatory governance (Caragliu et al. 2011, 68; Coe et al. 2001), which together with smart technologies "provide an opportunity for enhancing citizen participation in and influence over local decision making" (Hollands 2008, 315).Underlying assumption is that active participation of empowered citizens will work towards their own personal growth and in building stronger and smart communities: "including the importance of social learning, education and social capital for developing the smart city" (Hollands 2008, 309).

One way or another, it seems that technologically savvy and connected cities will be more efficient, productive and resilient cities and therefore ultimately become "happier cities," too (KamelBoulos et al. 2015, 3). However, many of such claims and promises are supported with little or no evidence. In fact, as we are going to

show in the following section, research shows that such technological determinism could in fact increase existing differences and even create new ones.

Smart City as another utopian vision in line of many 'technological fixes.'

A growing body of literature is currently addressing optimistic and uncritical views pertaining to Smart City as a very recent and still undefined concept. Technologically driven urban systems areoften characterizedas being utopian (Graham and Marvin 1996). Likewise the newest smart technological systems are described as elusive (Carvalho 2015, 45) and as a "technical fix or 'silver bullet' to somehow magically address complex and deep-seated social and political issues" (Crang and Graham 2007, 813), to mention just a few. Even just labeling cities and technological systems as 'smart' is problematic, as Hollands notes, because the premise implies positive and rather commonsensical development path since, after all, "which city, by definition, does not want to be smart, creative and cultural?" (Hollands 2008, 305)It is not the wishful thinking that presentsa challenge; a vision of inclusiveness and social inclusion at least serves as an aspiration and normative ideal. Graham warns that such overly generalized views are "hazardous" even more so because they are positive (Graham, 2004, 21). Is simply assuming consensus around impact and involvement of smart technologies problematic because it paints a false image of equality where it does not exist?

For a start, supporting technological networks are unevenly distributed, between the cities, and within the single city. Amin and Graham point to persistence of exclusion and differentiation of social strata amidst the promises of bridging inequalities:

"Even the most 'high-tech' of cities […] demonstrate that many social groups and geographical areas remain disconnected from the 'liberating' promise of new technologies for lack of funds, infrastructure, skills, equipment, even electricity." (1999, 28)

Suchsystems are therefore seen to work along current lines of social and digital inequalities "reproducing the status-quo" (Deakin 2011, 191) and by reinforcing urban fragmentation and divides (Crang and Graham 2007; Hollands 2008); social polarization (Hollands 2008, 311; Caragliu et al. 2011, 68) and discrimination (Bianchini& Avila 2014, 35); making those who are deprived of access and control over their selective processes eventually and inevitably "mute and invisible" (Crang and Graham 2007,797).The second point to note is that the overall increased dependency on contemporary technologies is putting pressure on those that are not able to, or who choose not to use them. We can see that even though the crucial role in place promotion and marketing of Smart Cities is precisely the vision of improved lifestyles of its citizens; social predicaments, if at all covered, are outnumbered by arguments for technological rationality and efficiency. (Bianchini& Avila 2014; Hollands 2008)

Likewise, many Smart City policies are taking the biased view towards technological efficiency, following up with the dominant top-down approach to implementation of technological systems, by laying down the infrastructure and ensuring access. (Phipps 2000; Shin 2009; Komninos et al. 2013; Carvalho 2015) As such, implementation ofubiquitous connectivity and universal access in U-Citiesin South Koreais taking the technical and economical perspectiveand lacks an awareness of underlying non-technical aspects and "social infrastructure." (Shin 2009, 522-523) This is not to state that top-down and "supply-push" approach to implementing technologies is not beneficial; it certainly contributes to overcoming issues with access and connectivity. However such approach has been shown as insufficient, (Carvalho 2015; Shin 2009) as it fails to include social and cultural aspects, the

specific concerns and contexts of use of the citizens involved: “Central planning often fails to create a city that is tailored to inhabitants’ needs and makes too many unjustified assumptions about what people want.” (Komninos et al. 2013, 130)As the top down approach proves to be insufficient to address the tension between main objectives and consequently between different stakeholder groups, recent research starts to recognize a need towards citizens’ participation in development and implementation processes. Researchers call for a mixture of both top-down and bottom-up approach to successfully balance diversity of stakeholders involved (Komninos et al. 2013, 121); andincluding citizens in the early stages of the development and design (Carvalho 2015; Pipps 2000; Shin 2009; among others).
Still the disproportion of seeming opportunities and benefits between users and nonusers mainly serves as an argument for further development of similar services but not for reconsideration of terms under which the same services operate. Assuming equal-for-all positive impact of smart technologies is downplaying and concealing negative effects of such development. (Hollands 2008; Graham and Marvin 2001) So, not only that uncritical positive premise obscures the reality of current digital and social inequalities, it makes a discussion around it more difficult and the rupture even deeper. Due to the imbalance of decision-making power during the developmental and implementation stages that fails to involve actual citizens, such technologies are eventually seen to only deepen existing social inequalities, and not overcome them, as the original mantra would state. The effects on citizens seen as a part of ‘unproductive sector,’ such as senior citizens, are even more critical to be investigated further.

Discourse surrounding elderly and smart technologies

The discourse of ‘‘silver surfer’’ (Selwyn 2004a, 370), connected and tech-savvy elderly, emerges under the common assumption that ICT use is “inherently useful and desirable activity throughout all sectors of society” including older adults. (Selwyn 2004a, 370, 381) The technological fixtures for elderly are again presented as a great opportunity for their wellbeing, ICTs in general seen to provide equal opportunities and digital inclusion for elderly (Ihm and Hsieh 2015; KamelBoulos et al. 2015; Plaza et al. 2011); reduce social isolation (KamelBoulos et al. 2015; Eggermontet al. 2006; Heart and Kalderon2013; Blit-Cohen and Litwin 2004, Karavidas et al. 2005); empower elderly, enabling democratic participation and social inclusion and engagement (KamelBoulos et al. 2015; Roupa et al. 2010; Obi et al. 2013; Blit-Cohena and Litwin 2004).Similarly, a number of academic studies show that the same technologies may work towards improving well-being and quality of seniors lives (KamelBoulos et al. 2015;Doukas et al. 2011; Obi et al. 2013; Heart and Kalderon2013; Roupa et al. 2010; Lam and Lee 2006, 178); successful and healthy ageing (Hernández-Encuentra et al. 2009, 229; Jung et al. 2010, 207); or simply towards meeting the need of ageing population. (KamelBoulos et al. 2015; Haux et al. 2008)
Even more dominant is the notion of necessity to address and mitigate economical burden of elderly-care for the future welfare system: “In the face of the aging population in Europe and all other continents with increasing costs for care for the elderly the necessity of all kinds of health-enabling technologies becomes evident.” (Haux et al. 2008, 80) Elderly care is commonly seen through the lens of cost effective and efficient health care (Eggermontet al. 2006, 203; Heart and Kalderon 2013; González et al. 2012) For one, ‘connected’ and tech-savvy elderly are seen as a predicament of self-care, and as such, it is expected of elderly in future to be more independent and to take care of themselves (Roupa et al. 2010)and engaging with, for instance, on-demand home healthcare and telemedicine (Roupa et al. 2010, 119) and using Internet for health related information (Karavidas et al. 2005;

Jung et al. 2010; Roupa et al. 2010, 119), followed by improving their mobility and transportation means (Roupa et al. 2010) and overall independent living (Doukas et al. 2011; Haux et al. 2008; Lam and Lee 2006, 178, Niehaves and Plattfaut 2014;González et al. 2012).
Despite the notions of empowerment,affordability, efficiency and utility, all lined up in promotion material of smart city technologies, adoption rates among elderly are currently seen as "problematic" (Heart and Kalderon2013, 211),to say the least, and the numbers of elderly using them are low (Eggermontet al. 2006; Jung et al. 2010; Niehaves and Plattfaut 2014; Blit-Cohen and Litwin 2004).This makes a task to bridge digital divide among elderly an "imperative," as Selwyn notes, (2004a, 381), and enforcing technological systemsa necessity(Doukas et al. 2011; Haux et al. 2008).Selwyn and Van Dijk alert to a common mistake to blame digital inequality to individual choices and lack of motivation, resulting to a general urge to adapt individuals to fit technologies and not vice versa (Selwyn 2004b; Van Dijk 2005).On contrary, seniors' ambivalence towards the technological system should be taken as an indicator of an inapt system in place, building "an attractive, interesting, or useful option for many older adults" instead (Selwyn 2004a, 382).The literature on elderly adoption of technologies, which is presented in the following section, shows that presumptions of elderly about technology are the main contributing factor to the eventual adoption. However, there is a great disparity between the developers idea of what smart technologies could and should do for elderly; and what elderly themselves feel about the benefits smart technologies could add to their lives.

Is there a place for elderly in smart city vision?

A common understanding among both policymakers and tech-developers, is that smart technological systems and devices need to be 'properly planned and implemented' if we want to see real benefits that such systems could bring (KamelBoulos et al. 2015; Doukas et al. 2011; Dogruel et al. 2015; Obi et al. 2013) The focus is on connectedness (Haux et al. 2008; González et al. 2012; Ihm and Hsieh 2015; Blit-Cohen and Litwin 2004), which usually translates in providing basic level of "universal access" (Selwyn, 2004a).More recently, there is a greater understanding that in order for technologies to be effective, people, in this case elderly, need to be willing (Heart and Kalderon 2013, 210) and need to "feel able to make use of such opportunities" (Selwyn 2004b, 347).Apart from general recommendations that we need to adapt technologies with elderly needs in mind (Eggermont et al. 2006, 216; Hernández-Encuentra et al. 2009, 238-239; Plaza et al. 2011, 1977); researchers recommend raising awareness about benefits(Hernández-Encuentra et al. 2009, 238-239; Niehaves and Plattfaut 2014, 722; Heart and Kalderon 2013, 219; Lam and Lee 2006, 178); proper training and social support to increase related skills (Obi et al. 2013; Blit-Cohen and Litwin 2004, 397; Hernández-Encuentra et al. 2009, 238-239; Niehaves and Plattfaut 2014, 722; Heart and Kalderon 2013, 219; Lam and Lee 2006, 178); and finally user-centered design efforts that cater specifically to elderly (Dogruel et al. 2015; Obi et al. 2013, 50).
Typically, user-centered studies present sampled senior citizens with a potential design option and works on its usability improvements, prompting further work on better system design and proper marketing (Dogruel et al. 2015, 1059).Such efforts are however shown to be insufficient, as the citizens are only included in the final stages, contributing with the decisions about adoption or non–adoption, and are not involved in the initial development and design stages(Aurigi 2008). Even when the system is particularly built to include disadvantaged groups of citizens, such as Bologna civic network discussed by Aurigi, efforts repeatedly translate in a top-down approach to infrastructure implementation and provision of access to

technologies, where citizens are expected to participate. Aurigi states that even though citizens are invited to comment on qualities of offered services, as "advanced users" and "information providers" (2008, 19), they are still not able to make any significant impact on the design and development of the system in place: "the definition of what the system should and should not be about still was a scarcely shared matter" (Aurigi 2008, 19). In this and similar cases user-needs are measured in terms of usability and performance of the given technologies, but technologies are seldom developed and crafted together with elderly considering their self-reported needs and wants.

More and more research point to the social issues that the same technologies transformations come along with; discussing for instance privacy and security, but also deepening of social inequality and the determining factors that might help in overcoming it. For a start research shows that elderly are heterogeneous group (Obi et al. 2013; Selwyn 2004a; Plaza et al. 2011; Ramón-Jerónimo et al. 2013) with deeply embedded both inter and intra-generational social and digital divide. Intergenerational divide shows that not only that elderly have different skills they also have different understanding of benefits pertaining to technological use than any other age group(Ling 2008). As an illustration, his research shows that elderly do not see smartphones as "harbinger of emancipation as with teens", or as"a symbol of status and power as with the middle-aged businessman." (Ling 2008, 336) Thus, in predicting their behavior and attitudes towards technologies we should not rely on theories and models based on younger population or even other marginalized groups. Secondly, even among elderly themselves there will be a diverse understanding of perceived benefits and wellbeing in general. (Plaza et al. 2011, 1985) In fact, according to Ramón-Jerónimo et al. elderly group of citizens is the most heterogeneous and diverse group of users (2013), in which demographic characteristics, age with accompanying cognitive and health status (Van Deursen and Helsper 2015; Ihm&Hseih 2015; Blit-Cohen and Litwin 2004; Eggermont et al. 2006) as well as socio-economic characteristics (such as income and education, previous job description, etc) determine the level of skills to use ICTs and therefore adoption rates. (Ihm and Hsieh 2015)

The socio-demographic characteristics alone, however, cannot explain the patterns of adoption and we need to take a closer look into attitudes and psychological factors (Dogruel et al. 2015; Ihm&Hseih 2015; Karavidas et al. 2005; Jung et al. 2010) Positive attitude towards new technologies, among many researchers is seen as the crucial factor that affects the intention of use – eagerness to learn and engage with ICTs at the first place. (Ramón-Jerónimo et al. 2013, 391; Heart and Kalderon 2013, 211-2; Van Deursen and Helsper 2015, 183; González et al. 2012, 586) Studies that look into attitudes of elderly towards new technologies identify factors such as perceived usefulness and perceived ease of use (TAM model in Heart and Kalderon 2013, 211-2); reporting self-described reasons such as lack of benefits, needs, relevance and similar (Selwyn, 2004a; Lin et al. 2012; Jung et al. 2010; Heart and Kalderon 2013) among the most frequent reasons for not engaging with ICTs. In contrast, it is shown that elderly are actually eager to see ICTs that would "enhance [their] quality of life (Eggermont et al. 2006, 216), and have capacity to learn new skills if they desire to do so (Blit-Cohen and Litwin 2004, 395). It is important to understand that the decision not to engage with ICTs is the personal choice of many senior citizens due to the perceived lack of relevance of current ICTs in their daily lives. (Lam and Lee 2006, 178) Unless the perception over personal relevance changes, there is a slim chance to got elderly fully embracing new technologies.

In fact, research shows that elderly do show great interest in learning new skills to both better themselves and to contribute to the society (González et al. 2012, 593).

The role of social context for elderly was reported extensively in research. Social surrounding, mainly family and friends, is seen as a valuable source of an exposure and encouragement to pick up new skills (Lam and Lee 2006, 198; Van Deursen and Helsper 2015, 183). González et al. note more positive attitude towards new technologies among elderly when there is a significant connection to their social lives, whether to maintain their existing social activities, to initiate new ones, or as a context in which they come to a contact with new technologies. (2012, 593) Moreover, due to the great need for social integration, (Lin et al. 2012, 85) in order to "maximize their processes of coexistence," (Pasqualotti et al. 2012, 443) and the strong levels of belongingness to a group, (Jung et al. 2010, 197) elderly will continue to develop further towards self-efficacy and sustainable use. Socialization, such as maintaining social contacts and participating in social activities, is also one of the main indicators for elderly well being. (Ihm and Hsieh 2015, 1126) The social aspects, particularly social context of learning and technology adoption, therefore should not be overlooked when planning and designing new technologies for elderly.

The way elderly understand and relate to technology largely depends on discursive construct of the very concept under which smart technology is promoted and implemented – the contemporary Smart City concept. Smart City discourse, an amalgam of ""'macro'' discourses of inherently beneficial, empowering, and ''magical'' new technologies" as Selwyn writes (Selwyn 2004a, 381), fails to address the tension between interest of economical sector and its stress on efficiency, productivity and competitive advantage, and the ""micro" everyday life perspective" (Selwyn 2004a, 381), actual day-to-day concerns of elderly. Digital inclusion is a matter of "sustained levels of engagement" among users, as Selwynremind us (2004b, 347), which largely depend on the "situational relevance of access to technology and information from the individual's point of view" (Selwyn 2004b, 350).It is thus vital to get to the core of elderly understanding of technology and how exactly it "fits into their lives and the effect it has upon them" (Dickinson and Gregor, 2006, 744).Selwyn suggests, before all, promoting more realistic expectations of ICTs use among elderly, which will be based on *self-reported impact, meaning and beneficial consequences* of ICTs. Including elderly in the design and the development process is not only the way to increase new technologies adoption among elderly, but also to address the multitude of needs within elderly group, Selwyn adds (2004a, 382; similarly in Plaza et al. 2011, 1987). However, only a few studies investigate appropriate bottom-up strategies pertaining to elderly involvement. In-depth analysis of seniors' current technological proficiency and adoption patterns may give useful guidelines for planning future strategies with elderly involvement in mind.

Conclusions and general recommendations for policymakers

Wile current Smart Nation promotion strategies do stress on the potential benefits for the elderly, these are mainly presented in terms of efficiency of the welfare system, economic viability, and similar, while actual seniors' anxiety and fears are seldom addressed. Hollands alerts about this underlying emphasis on this economic growth predicament, with the biggest challenge posed for smart city managers to "effectively balance the needs of the community, with both those of local government and the needs of business" (2008, 309). Balancing interest of different stakeholders under the economic development predicament becomes even more relevant in relation to elderly. Even if the empowerment and participation of citizens is possible, or as we can see even expected, the question is to what extent

marginalized groups, such as senior citizens, then have decision-making power within the system they do not belong to, or see fit to join?

Findings among current research on technology adoption underline necessity for seniors to see direct benefits in using technologies and especially smart features. Research results in this field show the prevalent negative attitudes towards smart technologies among seniors precisely due to a lack of need to 'join the bandwagon.'Smart City promotional strategies do not actually invite senior citizens to contribute to the system, seen rather as an economic burden than a productive force for the system. Quite contrary, the same policies are likely to be seen as irrelevant among elderly resulting in negative attitudes towards using available smart features and poor adoption. Elderly respondents repeatedly report that smart technologies are not cut for them, or that there is not much for them to contribute, seeing smart technology as unnatural, inauthentic, or irrelevant. Conveying the benefits of smart living should be a starting point to communicate policy. At this point, many of them do not see any real need to incorporate smart technologies into their regular ICT use. If the imagined beneficiary of these technologies can be changed from an efficient and productive Smart Nation (or Smart City) to a potentially Smart People, it might better motivate elderly to learn more about and make regular use of newer ICTs.

Existing research in this areapoints to the widening of both intergenerational and intra-generational digital divide, indicating that a large portion of elderly might be slow or even resistant to adopt and/or upgrade to smart technologies. Due to differences in socioeconomic status, cognitive and physical abilities, and most of all attitudinal factors, fewer and fewer elderly may report meaningful uses and outcomes in the future. From a policy point of view, this translates into the need for tailored policies that are appropriate for different classes of the elderly, instead of a homogenous, one-size-fits-all policy. It is recommended for policymakers to consider the different living conditions, needs and concerns within the elderly population and seek out their participation in the planning process.This is in line with the more recent research indicating that policymakers need to consider niche and bottom-up approach to their implementation strategies in place of current top-down approach, in order to encourage participation and adoption of new technologies among elderly. Policy makers should consider including elderly in the early stages of technological development and design; i.e. approach policy-making such that it could solicit and encourage the input of the elderly from the start.

Existing research in relation to elderly adoption of smart technologies mostly deals with technological improvements in area of usability. A few studies look how to include them in creative process of the technological development in order to achieve it. For that reason, it is necessary to engage in more on the grounds research to explore possible avenues for elderly participation, beyond usability studies. Available research results indicate that socialization and social learning are main motivating factors for introducing new technologies, acquiring necessary skills and further sustained use among elderly. In addition, staying in touch with family and friends is often reported among elderly as a good motivation to pick up and continue to use new technologies; therefore policymakers should further explore social context of learning and technology adoption for bottom up implementation strategies.

Our study aimed to open the discussion about potentials and issues in relation to elderly engagement with Smart Nation technology.Conclusions from the literature and current research in this field should serve as a starting point for more qualitative research on specific Smart City case studies, and to serve as a reference point for policy makers. By providing a picture of where the elderly are most likely to stand with respect to smart living, we hope for formulation and implementation of policies

that are actually applicable to the concerned segment of elderly, and which will stimulate more positive engagement. Whether Smart City vision will grow to be more than a prescribed and imposed technological system, and function as an all-inclusive and participatory system, depends on the future policies and development.

References

Accenture, Cisco, Inc and GSMA. 2011. "Smart Mobile Cities: Opportunities for Mobile Operators to Deliver Intelligent Cities"Accenture, Cisco, Inc and GSMA Report. Accessed Feb 2, 2016 http://www.gsma.com/connectedliving/wp content/uploads/2012/03/intelligentcitiesreport0411lores.pdf

Amin, Ash, and Stephen Graham. 1999. "Cities of Connection and Disconnection." InUnsettling Cities edited by John Allen, Doreen Massey and Michael Pryke, 7-48. London: Routledge.

Aurigi, Alessandro.2008. "Competing Urban Visions and the Shaping of the Digital City." Knowledge, Technology, & Policy 18 (1): 12-26.

Bianchini, David and IsmaelÁvila. 2014. "Smart Cities and Their Smart Decisions: Ethical Considerations."IEEE Technology and Society Magazine (Spring): 34-40.

Blit-Cohen, Edith and Howard Litwin. 2004. "Elder participation in cyberspace: A qualitative analysis of Israeli retirees."Journal of Aging Studies 18: 385–398.

Caragliu, Andrea, Chiara Del Bo and Peter Nijkamp.2011. "Smart Cities in Europe." Journal of Urban Technology 18 (2): 65–82.

Carvalho, Luís.2015. "Smart cities from scratch? A socio-technical perspective."Cambridge Journal of Regions, Economy and Society 8: 43–60.

Coe, Amanda, Gilles Paquet, and Jeffrey Roy. 2001. "E-governance and smart communities: a social learning challenge."Journal on Social Science Computer Review 19 (1): 80–93.

Crang, Mike and Stephen Graham. 2007. "Sentient Cities: Ambient Intelligence and the Politics of Urban Space." Information, Communication & Society 10 (6): 789-817.

Deakin, Mark. 2011. "The embedded intelligence of smart cities." Intelligent Buildings International, 3 (3): 189-197.

Dickinson, Anna, and Peter Gregor. 2006."Computer use has no demonstrated impact on the well-being of older adults."International Journal of Human-Computer Studies 64: 744–753.

Dogruel, Leyla, Sven Joeckel and Nicholas D. Bowman. 2015. "The use and acceptance of new media entertainment technology by elderly users: development of an expanded technology acceptance model." Behaviour& Information Technology 34 (11): 1052-1063.

Doukas, Charalampos, Vangelis Metsis, Eric Becker, Zhengyi Le, FilliaMakedon and IliasMaglogiannis. 2011."Digital cities of the future: Extending @home assistive technologies for the elderly and the disabled."Telematics and Informatics 28: 176–190.

Eggermont, Steven, Heidi Vandebosch and Stef Steyaert. 2006."Towards the desired future of the elderly and ICT: policy recommendations based on a dialogue with senior citizens."Poiesis& Praxis: International Journal of Technology Assessment and Ethics of Science 4 (3): 199-217.

Frost and Sullivan. 2014. Frost & Sullivan: Public-private sector collaboration opportunities key to successful Smart Cities. Frost and Sullivan Press Release (Nov 7). Accessed Feb 2, 2016 http://ww2.frost.com/news/press-releases/frost-sullivan-public-private-sector-collaboration-opportunities-key-successful-smart-cities/

González , Antonio, M. Paz Ramírez and Vicente Viadel. 2012."Attitudes of the Elderly Toward Information and Communications Technologies." Educational Gerontology 38 (9): 585-594.

Graham, Stephen and Simon Marvin. 1996. Telecommunications and the City: Electronic Spaces, Urban Places. London: Routledge.

Graham, Stephen and Simon Marvin. 2001. Splintering Urbanism: Networked Infrastructures, Technological Mobilities and the Urban Condition. London: Routledge.

Graham, Stephen.2004. "Beyond the 'Dazzling Light': From Dreams of Transcendence to the 'Remediation' of Urban Life: A Research Manifesto" New Media Society6: 16-26.

Hollands, Robert G. 2008. “Will the real smart city please stand up?” City: analysis of urban trends, culture, theory, policy, action12 (3): 303-320.
Hernández-Encuentra, Eulàlia, ModestaPousada and BeniGómez-Zúniga. 2009. ICT and older people: Beyond usability.” Educational Gerontology 35: 226–245.
Haux, Reinhold, Howe Jurgen, Michael Marschollek, MaikPlischke and Klaus-Hendrik Wolf. 2008. “Health-enabling technologies for pervasive health care: on services and ICT architecture paradigms.” Informatics for Health & Social Care 33 (2): 77 – 89.
Heart, Tsipi and EfratKalderon. 2013.“Older adults: Are they ready to adopt health-related ICT?” International Journal of Medical Informatics 82: 209–231.
Ihm, Jennifer andYuli Patrick Hsieh. 2015.“The implications of information and communication technology use for the social well-being of older adults.”Information, Communication & Society 18 (10): 1123-1138.
Jung, Younbo, Wei Peng, Meghan Moran, Seung-A Annie Jin, Margaret McLaughlin,Michael Cody, Maryalice Jordan-Marsh, Julie Albright and Merril Silverstein. 2010. Low-income minority seniors’ enrollment in a cybercafé: Psychological barriers to crossing the digital divide. Educational Gerontology 36: 193–212.
KamelBoulos, Maged N., Agis D. Tsouros, and ArtoHolopainen.2015. “'Social, innovative and smart cities are happy and resilient': insights from the WHO EURO 2014 International Healthy Cities Conference.” International Journal of Health Geographics14 (3).
Karavidas, Maria, Nicholas K. Lim and Steve L. Katsikas. 2005. “The effects of computers on older adult users.”Computers in Human Behavior 21: 697–711.
Komninos, Nicos, MarcPallot and Hans Schaffers. 2013.“Special Issue on Smart Cities and the Future Internet in Europe.”Journal of the Knowledge Economy4:119–134.
Lam, Jolie C.Y. and Matthew K.O. Lee. 2006.“Digital Inclusiveness—Longitudinal Study of Internet Adoption by Older Adults.” Journal of Management Information Systems 22(4):177-206.
Lin, Cecilia I. C., Wen-hui Tang and Feng-Yang Kuo. 2012. "Mommy Wants to Learn the Computer": How Middle-Aged and Elderly Women in Taiwan Learn ICT Through Social Support.” Adult Education Quarterly 62 (1): 73-90.
Ling, Rich. 2008. “Should We Be Concerned That the Elderly Don’t Text?” The Information Society 24 (5): 334-341.
Niehaves, Björn and Ralf Plattfaut. 2014. “Internet adoption by the elderly: employing IS technology acceptance theories for understanding the age-related digital divide.”European Journal of Information Systems 23: 708–726.
Obi, Toshio, Diana Ishmatova and Naoko Iwasaki.2013. “Promoting ICT innovations for the ageing population in Japan.” International Journal of Medical Informatics 82: 47–62.
Pasqualotti, Adriano, Dante Augusto CoutoBarone and Johannes Doll. 2012. “Communication, Technology and Ageing: elderly, senior citizen groups and interaction process in the information age.” Saúde Soc. São Paulo 21 (2): 435-445.
Phipps, Linda. 2000. “New communication technologies—a conduit for social inclusion.” Information, Communication & Society 3 (1): 39–68.
Plaza, Inmaculada, Lourdes Martín, Sergio Martin and Carlos Medrano.2011. “Mobile applications in an aging society: Status and trends.”The Journal of Systems and Software 84: 1977–1988.
Piro, Giuseppe, IlariaCianci, Luigi Alfredo Grieco, GennaroBoggia, and PietroCamarda. 2013.“Information centric services in Smart Cities.”The Journal of Systems and Software 88 (2014): 169-188.
Ramón-Jerónimo, María A., BegoñaPeral-Peral and Jorge Arenas-Gaitán.2013.“Elderly Persons and Internet Use.”Social Science Computer Review 31(4): 389-403.
Roupa, Zoe, MariosNikas, Elena Gerasimou, VasilikiZafeiri, LampriniGiasyrani, EunomiaKazitori and PinelopiSotiropoulou. 2010. “The use of technology by the elderly.” Health Science Journal4 (2): 118-126.
Selwyn, Neil. 2004a.“The information aged: A qualitative study of older adults’ use of information and communications technology.”Journal of Aging Studies 18: 369– 384.

Selwyn, Neil. 2004b. “Reconsidering political and popular understandings of the digital divide.” New Media & Society6(3):341–362.
Shin, Dong-Hee. 2009.“Ubiquitous city: Urban technologies, urban infrastructure and urban informatics.” Journal of Information Science 35 (5): 515–526
Van Deursen, Alexander JAM and Ellen J Helsper. 2015. “A nuanced understanding of Internet use and non-use among the elderly.” European Journal of Communication 30 (2): 171-187.
Van Dijk, Jan A.G.M. 2005. "A Framework for Understanding the Digital Divide." In The Deepening Divide: Inequality in the Information Society, 9-27. Thousand Oaks: SAGE.
Yin, ChuanTao, Zhang Xiong, Hui Chen, JingYuan Wang, Daven Cooper and Bertrand David.2015.“A literature survey on smart cities.”Science China Information Sciences 58 (Oct):1-18.

An Evaluation of the Japanese Quasi-Zenith Satellite System LEX Augmentation Corrections in Vietnam

HIEP HOANG-VAN, TU NGUYEN THI THANH, VINH LA THE

NAVIS Centre, Hanoi University of Science and Technology, Vietnam

Abstract

Recently, Japan Aerospace Exploration Agency (JAXA) provides Quasi-Zenith Satellite System (QZSS), a regional satellite system covering East Asia and Oceania region. The objective of QZSS is to improve the availability as well as the performance of GNSS system. Besides navigation signals, QZSS also transmits L-band Experimental signal (LEX) for augmentation corrections including orbit and clock information that support Precise Point Positioning (PPP). In this paper, a method for decoding LEX signal is first presented. Next, the availability of the LEX signal and the quality of the broadcasted correction messages for PPP solutions are evaluated in Vietnam. The results of PPP using LEX signal have been compared to those using augmentation corrections from International GNSS service (IGS) in both static and kinematic positioning modes. Experimental results conducted in Hanoi, Vietnam show the feasibility of using LEX signal to deliver high accuracy positioning service to Vietnamese GNSS users.

Index Terms: Quasi-Zenith Satellite System (QZSS), L-band Experimental signal (LEX), Precise Point Positioning (PPP) Evaluation.

Introduction

Precise Point Positioning has been becoming a hot topic in GNSS field recently. This technique provides positioning solutions at centimeter-level without the need of having any nearby ground GNSS reference station through the use of orbit and clock corrections. Therefore, PPP is really useful at remote regions where ground-based reference stations are sparse or even unavailable. However, PPP service providers require a communication link to deliver the fixed satellite positions and clocks to PPP users. The communication links can be generally divided into two types including (1) terrestrial links such as Internet and (2) space-based means such as satellites. In the former type, the International GNSS Service (IGS) is an example [GNSS service 2015]. Currently, IGS supports two GNSS, GPS and the Russian GLONASS. On the other hand, the Quasi-Zenith Satellite System (QZSS) provided by JAXA, Japan is an example of the space-based type [MADOCA 2015]. In particular, the L-band Experimental signal broadcasted by QZSS includes necessary augmentation corrections for PPP users. Compare to the conventional approach, the terrestrial communication links, the space-based means may have following advantages: (1) the corrections transmission can be received by a large numberof PPP users, and (2) the receivers can compute the precise position by itself without any support from outside.

The objective of QZSS is to improve not only the availability but also the performance of current GNSS over Japan and neighboring Asia Oceania countries. Many researches have been proposed to evaluate the quality of the current satellite orbit and clock corrections broadcasted by QZSS in the LEX signal. However, almost all of the evaluation experiments were conducted in Japan, some others were done in Australia where the QZSS satellites ground track also cover. Vietnam is quite close to Japan, therefore the QZSS satellite can be viewed with a high elevation angle, a good quality of received signal from the QZSS satellite is

foreseen. However, to the best of our knowledge, no evaluation of QZSS LEX signal was conducted in Vietnam. This study aims to thoroughly evaluate the QZSS LEX signal in order to confirm the availability and the performance of the signal to Vietnamese users.

There are several types of LEX messages, some of them are only available in Japan (include tropospheric corrections), some messages are now in the testing phase and not available for public users [JAXA 2015]. JAXA has begun a test transmission of MADOCA-LEX message in April 2013 [JAXA 2013,Takasu and Tomoji 2013]. This is another type of correction messages that are delivered in LEX signal and has a wide-area validity. The difficulty is that current commercial receivers have no ability to decode the MADOCA-LEX signal. Therefore, we propose to use a software define GNSS receiver to decode the LEX message and to compute the position. The software GNSS receivers are currently widely used due to their configuration flexibility and the ease of use [James and Tsui 2005,David and Jame 2000]. Further, in this study, we have also confirmed the capability of LEX signal and MADOCA-LEX message using post processing PPP in both static and kinematic scenarios.

The rest of this paper is organized as follows. In Section II, we discuss about the related work. Section III gives an overview of the QZSS and the LEX signal. In Section IV, a method for decoding LEX signal is presented. Next, we demonstrate the experimental results in Section V. Finally, conclusions and future work are provided in Section VI.

Related Work

In IGS workshop 2010, Takasu introduced RTKLIB for real-time PPP using IGS real-time satellite orbit and clock corrections [Takasu 2010]. The accuracy for PPP solution is impressive, in particular, the accuracy for PPP static is reported as 7.5 cm for N-Sand 10.6 cm for E-W respectively, after 60 minutes of convergence time. In this paper, we used RTKLIB software version 2.4.2 as a tool to process PPP using IGS augmentation. The results are used as baselines for comparison with PPP using QZSS LEX augmentation.

Kitamura et al. proposed to use QZSS to improve the availability and the accuracy of the Multi-GNSS positioning in urban canyon environments [Kitamura 2014]. They showed that the conventional Multi-GNSS is problematic because of insufficient number ofsatellites for positioning computation due to the fact that one satellite must be always defined as the master in each GNSS. The authors proposed to use QZSS as the sole master satellite. The GNSS used in their method including GPS, GLONASS, Galileo, Beidou, and QZSS. Static evaluation tests conducted in open-sky and narrow-sky environments show the efficiency of their method. In this study, we focus on another application of QZSS. Rather than solve Multi-GNSS problem, we focus on PPP using the correction messages broadcasted by QZSS LEX.

Suzuki et al. introduced a novel method for decoding LEX messages using software GNSS receiver [Suzuki 2014]. To acquire the LEX signal, the authors proposed to use not only the LEX but also the L1CA signal which is transmitted simultaneously with theLEX signal in QZSS. In particular, the L1 CA signal is first acquired and tracked to estimate the accuracy code phase and the Doppler frequency every 1 ms. Next, the original LEX code phase, i.e., the non-shifted phase (note that the LEX uses code shift keying modulation) of the LEX signal can be resolved by synchronizing the navigation bit overlaid in L1CA code. Derive from this idea, this study implements a method to acquire and decode the LEX signal received in Vietnam.

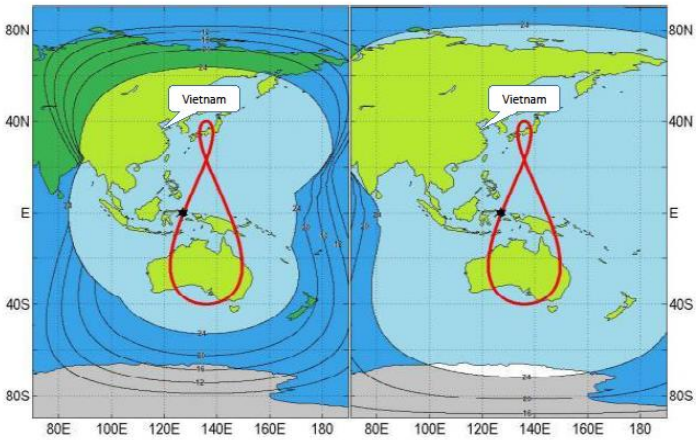
Choy et al. introduced an evaluation of a real-time PPP application using correction messages broadcasted by LEX signal in Australia [Choy 2015]. The results show

that the availability of the LEX in Australia is quite good, about 60% when the QZSS satellite elevation is at 30 degree and up to above 90% when the elevation is above 40 degree. In addition, real-time PPP solution has been evaluated in both fixed-point and moving vehicle tests. For the fixed-point test, centimeter-level position accuracycan be obtained after two hours of convergence. For the kinematic mode, the decimeter-level accuracy can be reached after approximately 180 minutes left for PPP solutions to converge. Our aims is similar to their work but we evaluate the PPP application using LEX in Vietnam. Furthermore, we also present a method to decode the LEX signal which was not introduced in Choy et al's work.
A Joint project between the Australian Cooperative Research Centre for Spatial Information (CRCSI) and JAXA has kicked off to assess the feasibility of using LEX signal to provide precise positioning service in Australia [Harima 2014]. In particular, they introduced "Australian-generated LEX corrections" prototype. The process is as follows: (1) correction messages collected in Australia based on IGS and CNES real-time products are packaged into LEX messages using the MADOCA-LEX message's format; (2)the packaged LEX messages are then transmitted to QZS master control station in Japan for transmission by QZS-1; (3) the messages will be received in Australia and used to compute PPP solutions. The purpose of the joint project is to augment the current correction messages in order to enable PPP Ambiguity Resolution (PPP-AR) [M. Ge 2007].

QZSS and LEX Signal

QZSS

Figure 3: Ground track of QZSS satellites (source CRCSI), the contour lines show the number of hours per day the signal will be available in 2018

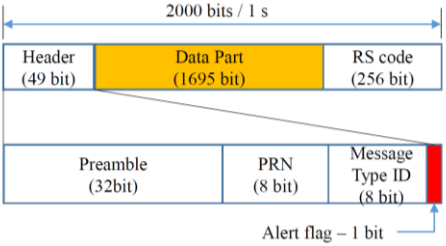


QZSS is a regional satellite navigation system provided by Japanese. The objective of QZSS is to enhance the availability and performance of the current GNSS positioning. According to the IS-QZSS [JAXA 2015], QZSS consists of QZSS Space Segment (SS) and QSZZ Ground Segment (GS). In 2018, the SS will consist of three satellites placed in Highly Inclined Elliptical Orbits and one placed in geostationary orbit. All the satellites will have the same "figure-8" orbit ground track

passing over Japan, Southeast Asia and Oceania countries. Figure 1 shows the ground track of QZSS as well as the number of hours per day the signal will be available in different regions. It is likely Vietnam will have 24 hours access to the QZSS signal. However, at this moment, only one satellite named MICHIBIKI is available, it was launched in September 2010.
MICHIBIKI transmits two types of signals: (1) navigation signals compatible with the modernized GPS signal such as L1CA, L1C, L2C, L5; and (2) augmentation signals including L1-SAIF and the LEX. The objective of the augmentation signals is to realize precise positioning (centimeter level).

LEX Signal

Figure 4: LEX message structure



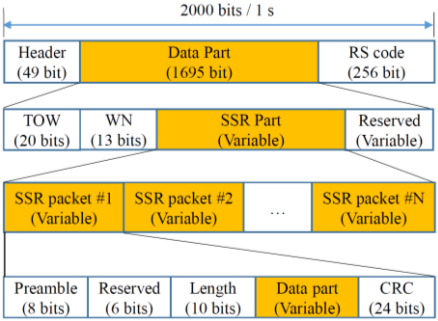
The L-band Experimental Signal is an augmentation signal transmitted on 1278.75 MHz carrier, same frequency as that of Galileo E6b signal. The LEX is modulated by Bi-Phase Code Shift Keying (BPSK). The LEX signal is generated by interleaving two 2.5575 MChip/s bit streams: (1) a PRN short code modulated by means of code shift keying by the Reed-Solomon encoded navigation message, and (2) a PRN long code modulated by squarewave with 820 ms period. The code shift keying will make a right circular shift to the phase of the PRN short code, the number of chips to be shifted representing in 8-bit encoded Navigation message symbol.
Figure 2 shows the structure of LEX message. The message length is 2000 bits: a 49 bits header, 1695 bits of data, and 256 bits for Reed Solomon error correction. It takes one second for one message transmission. In particular, 32 first preamble bits, “1A, CF, FC, 1D", are located at the beginning of each message. Next 8 bits indicate the PRN transmitting the message (193 for QZS-1, and 194 to 196 for three future QZSS satellites). Reed Solomon (255, 223) encoding is applied into 1744bits of navigation message (preamble, PRN, message type, 1 alert flag bit and the data part) to form 250 symbols of LEX message.
There are many message types of LEX as shown in table 1, one of them is MADOCA-LEX message, the message type 12. MADOCA stands for Multi-GNSS Advanced Demonstration tool for Orbit and Clock Analysis. As showed in the name, MADOCA provides the satellite orbit, clock corrections, code bias, and the user range accuracies (URAs). MADOCA uses the state-space representation (SSR) [Wübbena 2005] format for PPP. In SSR format, the errors are modeled and transmitted individually. The data partin LEX message (1695 bits) is divided into several SSR packets with variable length, as shown in Fig. 3.
As noted in the IS-QZSS, when the MADOCA is broadcasted, other types of test messages (type 10 and 11) will not be transmitted simultaneously with the message

type 12. In other words, to receive MADOCA signal, we have to check the transmission schedule onJAXA home page [QZ-vision].

Table 1: Message Types of LEX

Message Type		Content	Note
10-19	10	Signal Heath, URA, Ephemeris 1	
	11	Signal heath, Ephemeris, clock, ionopheric correction	
	12	Orbit, clock, URA, SV code bias	MADOCA-LEX
	13-19	Reserved	
20		GSI experiment	
21-255		Reserved for other agencies	

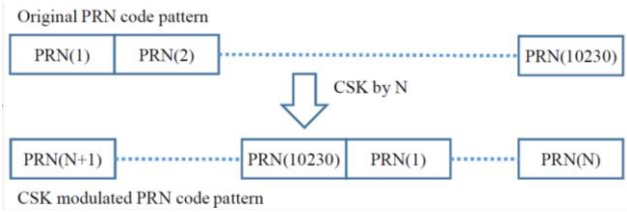
Figure 5: MADOCA-LEX message structure



LEX Signal Decoding Scheme

Herein, we present a method for decoding LEX signal using software GNSS receiver technique. To decode the LEX messages, we first need to understand how the navigation messages are encoded and modulated in LEX. As described in IS-QZSS, the original navigation message of LEX is 1744-bit data bit string per second (218 symbols, 8 bits of bit-stream comprises one symbol) including preamble, PRN, message type ID, alert flag, and data section. Reed-Solomon (255, 223) is applied in order to add 32 parity symbols(256 bits) to the original 218 symbols to form 250 symbols data bit stream. These 250 symbols (2000 bits) are then input to CSK modulator, which shifts the code phase of PRN short code by the number of chips defined in the 8-bit symbol every 4 ms. Figure4 shows how the CSK modulation works with short code.

Figure 6: Code shift keying modulation



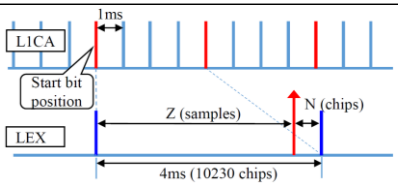
The LEX messages can be decoded if we can find 8-bit encoded navigation message symbol, denoted as N, which is modulated in PRN short code every 4 ms. To achieve this, we have to estimate the LEX code phase, i.e., how many chips the PRN short code has been shifted. To find N, we clearly need to track the short code. However, to track the short code, a symbol decision procedure is required. This leads to a problem to decoding the LEX using only the LEX signal. This paper presents a method to decode the LEXmessages with the aid of tracking results from L1CA signal which is simultaneously broadcasted from QZSS. Algorithm 1 shows the pseudo-code of our software receiver for LEX decoding. The algorithm in detail is as follows:

Figure 7: Computation of N - navigation message symbol

Algorithm 1: LEX messages decoding process.

Data: Digitized data of L1CA and LEX signal (output of front ends)
Result: Original LEX messages

```
while TRUE do
    [doppler_freq_acq, code_phase_acq, acq_OK] ⇐ Acquire_L1CA_by_FFT();
    if acq_OK then
        [sample_skip, doppler_freq_FLL, code_phase_FLL, FLL_OK] ⇐ Track_L1CA_FLL_DLL(doppler_freq_acq, code_phase_acq);
        if FLL_OK then
            [doppler_freq_PLL, code_phase_PLL, PLL_OK] ⇐ Track_L1CA_PLL_DLL(sample_skip, doppler_freq_FLL, code_phase_FLL);
            if PLL_OK then
                doppler_freq_LEX ⇐ Compute_doppler_freq(doppler_freq_PLL);
                [code_phase_shifted_LEX, acq_OK] ⇐ Acquire_LEX_by_FFT(code_phase_PLL);
                if acq_OK then
                    N ⇐ Compute_symbol_value(code_phase_shifted_LEX);
                    encoded_LEX_message ⇐ Form_Lex_Message(N);
                    original_LEX_message ⇐ Reed_Solomon_Decode(Encoded_LEX_message);
                    parameters ⇐ Extract_Parameters_from_Message(Original_LEX_message);
```



– First, the QZSS L1CA signal is acquired based on fast Fourier transform (FFT) method to find the coarse Doppler frequency and the coarse code phase of the signal. These parameters will be used as initial values for the next process, the tracking loop. The outputs of tracking process are the fine Doppler frequency and the fine code phase of the L1CA signal every 1 ms. Since the L1CA and LEX are transmitted simultaneously, the beginning of navigation bit in L1CA and that in original LEX (non-shifted LEX) must be aligned. In other words, we can find the start bit of original LEX message by tracking L1 signal.
- Second, we can compute the Doppler frequency of the LEX signal based on the Doppler frequency of the L1CA as follows:

$$f_{D,LEX} = f_{D,L1}\frac{f_{carr,LEX}}{f_{carr,L1}} \qquad (1)$$

where $f_{D,L1}$ is the Doppler frequency of L1CA, $f_{carr,LEX}$ and $f_{carr,L1}$ are signal frequencies of the LEX and L1CA signal, they are 1278.75 and 1575.42 MHz respectively.
Figure 5 illustrates the computation process of N, 8-bit navigation message symbol. Note that the beginning of navigation bit of original LEX, i.e., non-shifted LEX, is aligned with that of L1CA. However, due to the CSK modulation, the code phase of LEX is biased a number of samples, denoted as Z in Fig. 5. This bias can be

calculated by acquiring the LEX signal with a given Doppler frequency computed from Eq. 1. By determining the value of Z, theLEX navigation message symbol N can be computed as following equation:

$$N = \frac{f_{sample,LEX} \times T_{LEX} - Z}{f_{sample,LEX} \times T_{LEX}} \times LC_{LEX} \quad (2)$$

where $f_{sample,LEX}$ is the sampling frequency of the GNSS radio-frequency front end, T_{LEX} is the period of LEX short code (4 ms), and LC_{LEX} is the chip length of the LEX (10230).

- Some of the LEX navigation message symbols that we calculated above may have errors due to the sensitive of the acquisition process. Fortunately, these errors can be corrected by Reed-Solomon code. The Reed-Solomon code used in LEX allows us to correct up to 16 symbols. If the number of error symbols is exceed 16, the message will be discarded. This step will decode the encoded messages to get the original messages.

– Finally, all the necessary parameters such as satellite orbits and the clock corrections can be extracted from the original navigation messages.

Evaluation of the LEX signal in Vietnam

As mentioned above, the main contribution of this work is to evaluate the LEX signal in Vietnam in order to provide valuable information to Vietnamese GNSS research community. We first evaluated the quality of LEX signal in Hanoi, Vietnam by computing the C/N0. The performance of PPP solution using MADOCA-LEX messages are then evaluated in both static and kinematic environment. For simplicity, all the experimental processing was done in RTKLIB software version 2.4.2 [Takasu 2010], the rtkpos in particular. In the static mode, the results of PPP using MADOCA-LEX were compared to those of PPP using IGS. The known coordinates of testing stations were used as ground truth to evaluated PPP static estimates. In the kinematic mod, we applied RTK-GNSS technique to track the position of the moving receiver [Kaplan and Hegarty 2005,Leick 2004]. The base station is located in Ta Quang Buu building which is very close to the kinematic testing area, as shown in Fig. 7. This ensures the centimeter-level accuracy of the RTK solution. These results were used as ground truth for PPP kinematic tests.

The quality of the LEX signal

To confirm the quality of the LEX signal in Hanoi Vietnam, we computed the C/N0 of the signal. Figure 6 shows the C/N0 during 5 minutes of signal received on 3rd December 2015. We can see that the signal power is very high, i.e., the C/N0 is almost 50 dB-Hz, which is enough to acquire and track the signal. This result demonstrates the feasibility of the LEX signal to Vietnamese GNSS users.

Figure 8: CN0 during 5 minutes test the LEX signal

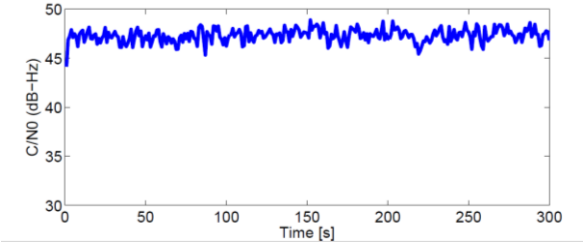
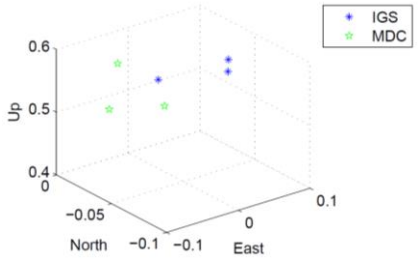
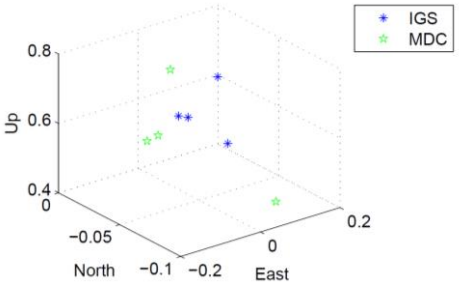


Figure 9: East, North, and Up components of positioning error in meter for the PPP static test at three reference stations

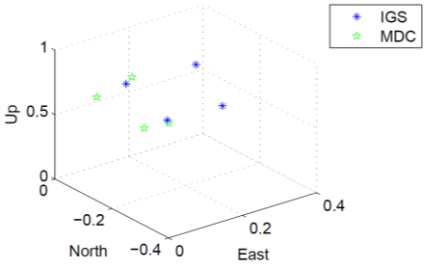
(a) VHHG, PPP static test in three different days (270, 290, 292)



(b) TQBL, PPP static test in four different days (270, 290, 292, 325)



(c) DHCG, PPP static test in three different days (270, 290, 292, 325)



PPP static test using MADOCA-LEX products

We have our testing CORS network, the NAVINET [Navinet], covering the region of Hanoi city which consists of 5 reference stations with known coordinates. These stations store RINEX (Receiver Independent Exchange Format) files every day. The data used for PPP processing was collected from three of the reference stations. The name and coordinates of these stations are presented in Table 2. For each station, several tests of PPP processing using IGS and MADOCA-LEX products were undertaken. The final computed positions were then compared to the known

positions to estimate the positioning error. Figure 6 shows the East, North and Up components of positioning error for several tests at three stations. Wecan see that the results of MADOCA-LEX solution are similar to those of IGS solution. In addition, both of solutions can reach centimeter-level of accuracy in horizontal plane. However, the error of the Up direction in all stations are unexpected high, e.g., in the worst case, the error of MADOCA-LEX and IGS are 66 cm and 60 cm, respectively. This bias is because we did not provide the corrected height of the antennas installed at the rooftop of reference station buildings. Nevertheless, with a similar performance compare to IGS in static environment, we can conclude that the MADOCA-LEX messages can also be used in Vietnam for PPP application with fixed-point position calculation.

PPP kinematic test using MADOCA-LEX products

We conducted the kinematic test on 3rd February 2016 from 08:30 to 08:45 UTC in the square in front of C1 Building, Hanoi University of Science and Technology, Vietnam. In particular, we walked around a fountain in the square two times to make circles. Wereceived the GPS signal using Trimble receiver with antenna Trimble zephyr geodetic model 2. All received raw data was stored in a laptop PC for post processing later. Figure 7 shows the area of the PPP kinematic testing.

To prepare the ground truth for evaluating the results of PPP using MADOCA-LEX corrections and IGS products, we use RTK method to track the position of moving receiver. The station named TQBL was selected as a base station for computing RTK. Since the TQBL station is very close to the testing area, the RTK results are expected to be centimeter-level. The coordinate of TQBL was also used to compute the positioning error of the kinematic test.

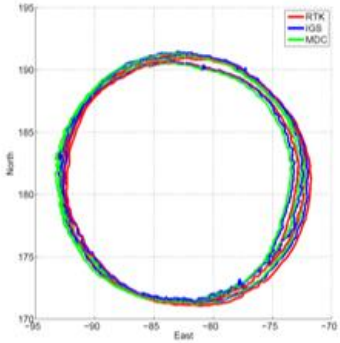
Figure 12 presents the horizontal positioning error of the PPP kinematic test. From this figure, the performance of MADOCA-LEX is qualitatively similar to that of IGS products and RTK method. In particular, the results show circlesrepresenting the orbit of the moving receiver in practice. For quantity analysis, we compare the results obtained by MADOCA-LEX and IGS to the ground truth (RTK) by computing the differences among them. Figure 12 illustrates the results of subtraction between IGS, MADOCA-LEX with the RTK in east, north, and up direction. We can see the accuracy of MADOCA-LEX is better than IGS in North direction but worse in East direction. For Up direction, the performances of two solutions are the same. Therefore, it is hard to determine which one, MADOCA-LEX or IGS is better in term of accuracy. For precision, the MADOCA-LEX seems to be better due to the smooth of variation. As a result, the standard deviations (SDs) of the difference between MADOCA-LEX and RTK in all directions are smaller than those of IGS and RTK, as shown in Table 3. The SDs in case of MADOCA-LEX are about 6 cm, 6 cm, and 20 cm for East, North, and Up direction respectively, whereas the SDs in case of IGS are 8 cm,9.6 cm, and 30 cm for corresponding directions.

These experimental results indicate the feasibility of MADOCA-LEX messages in Vietnam for the PPP processing in both static and kinematic modes.

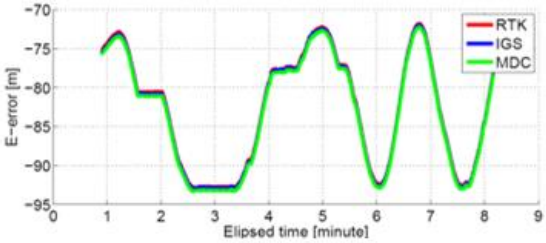
Figure 10: Kinematic testing area

Figure 11: Horizontal positioning error and the time-series positioning error in the east, north, and up directions

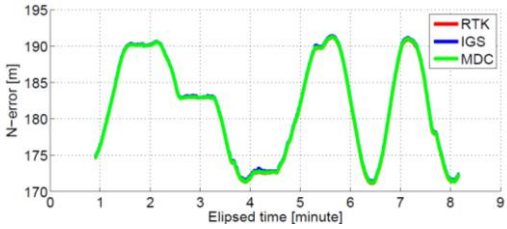
(a) Horizontal positioning error in meter



(b) East Error



(c) North Error



(d) Up Error

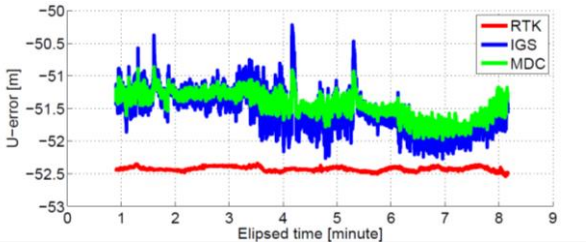
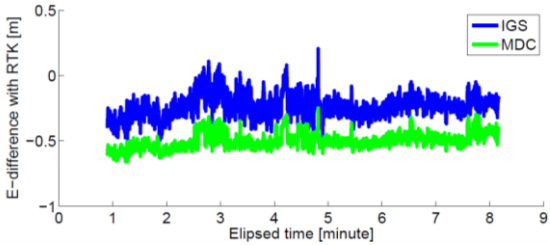
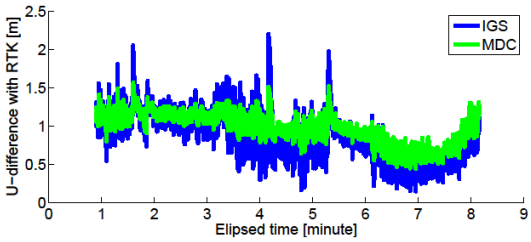


Figure 10: Difference between IGS, Madoca with the 'ground truth' RTK

(a) East difference



(b) North difference



(c) Up difference

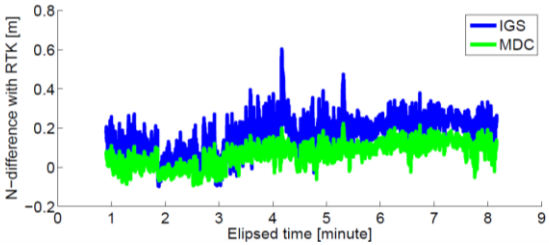


Table 2: Coordinates of three reference stations

Station Name	x	y	z
TQBL	-1626343.5785	5730606.2809	2271881.4349
VHHG	-1629707.7685	5730163.2216	2270485.6738
DHCG	-1625304.0865	5731515.5156	2270233.6821

Table 3. Standard deviation of subtractions (IGS-RTK and Madoca-RTK)

Method	East [m]	North [m]	Up [m]
IGS-RTK	0.0865	0.0962	0.3178
MDC-RTK	0.0608	0.0611	0.2059

Conclusion

This study evaluated the availability as well as the performance of LEX signal in Vietnam. We introduced the Quasi-Zenith Satellite System, the LEX signal, and the MADOCA-LEX message. We also presented a method to decode the LEX signal using software receiver approach. By tracking L1CA signal which is transmitted simultaneously with the LEX signal, the ambiguity of the original LEX short code phase can be resolved. Consequently, the LEX messages can be decoded. The performance of PPP using MADOCA-LEX messages was evaluated in static and kinematic scenarios. Experimental results draw following conclusions: (1) the LEX signal is available in Vietnam with a good quality, i.e., high C/N0; (2) the performance of MADOCA-LEX signal is similar to that of IGS products for PPP solutions in both static and kinematic test.

Several future challenges remain. First, we just evaluated the PPP solutions using LEX messages in off-line mode (post processing), the evaluation of real-time PPP using LEX in Vietnam is still an open issue. Second, all experiments presented in this study were carried out in open sky conditions. In the future, we will do more experiments in the urban environment.

Acknowledgments

This work was partially supported by Hanoi University of Science and Technology, research project No. T2015 - 011.

References

[GNSS service 2015] International GNSS Service, http://igscb.jpl.nasa.gov/, Accessed 2015.
[MADOCA 2015] QZSS MADOCA Real-Time Products, http://qzss.go.jp/en/, Accessed 2015.
[JAXA 2015] Japan Aerospace Exploration Agency, “Quazi-Zenith Satellite System Navigation Service, Interface Specification for QZSS (IS-QZSS) Version 1.6” Technical report, November 28, 2014.
[JAXA 2013] Japan Aerospace Exploration Agency, “MADOCA-LEX format (Provisional)”, 2013.
[Takasu and Tomoji 2013] Takasu, Tomoji. 2013. “Development of Multi-GNSS Orbit and Clock Determination Software MADOCA.” *The 5th Asia Oceania Regional Workshop on GNSS*. December 1-3.
[James and Tsui 2005] James B.Y. and Tsui.2005.*Fundamentals of Global Positioning System Receivers: A Software Approach*.Second Edition, Wiley.
[David and Jame 2000] David M. Lin, and James B.Y. Tsui. 2000."Comparison of Acquisition Methods for Software GPS Receiver," *Proc. of ION GPS 2000*.
[Takasu 2010] Takasu, T. (2010). RTKLIB: An Open Source Program Package for GNSS Positioning, http://www.rtklib.com/, Accessed 2015.
[Kitamura 2014] Kitamura, M., Ota, T., and Amano, Y. 2014."Improving availability and accuracy of multi-GNSS positioning using QZSS." *Institute of Navigation* 3: 2341-2345.
[Suzuki 2014] Suzuki, T., Kubo, N., and Takasu, T.. 2014. "Evaluation of Precise Point Positioning Using MADOCA-LEX via Quasi-Zenith Satellite System," *Proceedings of the 2014 International Technical Meeting of The Institute of Navigation*, San Diego, California, January, 460-470.
[Choy 2015] Choy, S., Harima, K., Li, Y., Choudhury, M., Rizos, C., Wakabayashi, Y and Kogure, S. 2015. "GPS Precise Point Positioning with the Japanese Quasi-Zenith Satellite System LEX Augmentation Corrections."*Journal of Navigation*, July, 68 (4): 769-783.
[Harima 2014] Harima, Ken, Choy, Suelynn, Wakabayashi, Yaka, Kogure, Satoshi, Rizos, Chris, “Transmission of Augmentation Messages for Precise Point Positioning Utilizing Japanese QZSS LEX Signal," *Proceedings of the 27th International Technical Meeting of The Satellite Division of the Institute of Navigation (ION GNSS+ 2014)*, Tampa, Florida, September 2014,2467-2477.
[M. Ge 2007] M. Ge, G. Gendt, M. Rothacher, C. Shi, and J. Liu. 2007 “Resolution of GPS carrier-phase ambiguities in Precise Point Positioning (PPP) with daily observations," *Journal of Geodesy* 82: 389-399.
[Wübbena 2005] Wübbena, G., Schmitz, M. and Andreas, B. 2005. "PPP-RTK: Precise Point Positioning Using State- Space Representation in RTK Networks."*Proceedings of the ION GNSS 18th International Technical Meeting of the Satellite Division*. Long Beach, CA. 13-16 September 2005.
[QZ-vision] http://qz-vision.jaxa.jp/USE/en/lex/.
[Kaplan and Hegarty 2005] Kaplan, E. and Hegarty, C. 2005. *Understanding GPS: Principles and Applications*. Artech House, Boston.
[Leick 2004] Leick, A. 2004. *GPS Satellite Surveying*, John Wiley & Sons, Inc., Hoboken, New Jersey, USA.
[Navinet] Navinet, http://navisurvey.cloudapp.net:53059/.

CITY113: A Mobile Application to Improve Public Participation in Surabaya City Development for Better Governance

NUR AINI RAKHMAWATI, IRMASARI HAFIDZ,
RENNY P. KUSUMAWARDHANI, RADITYO P. WIBOWO

Department of Information Systems, Faculty of InformationTechnology, Institut Teknologi Sepuluh Nopember, Indonesia

Paper Abstract

Nowadays, a citizen may report about the condition of his/her neighborhood; a broken bridge, information report about a damaged road, news about traffic or about poverty in their neighborhood. Most citizens definitely have some concerns regarding the things about his/her city but do not have any access to contact or report to the official government. There is the need for an application which enables citizens in an effective and efficient ways to participate in local government. This can be done by addressing their concerns and forwarding those concerns to official government in a single citizen-reporting management systems.

To date, governments in cities begin to explore the usage of digital tools; social media and apps are quickly becoming a platform for citizen journalism and public engagement. Therefore, we employ two methods for building a single citizen-reporting management systems: crowdsourcing and gamification. We gather the citizen reports from social media accounts: @sapawargasby, @infosurabaya and @ e100ss that serve as a means for delivering netizen report related to the public service in Surabaya. An annotation method was implemented over the data to categorize the data based on frequently keywords and the government agencies. The outcome of this process is being delivered to a mobile application called City113. In addition, the City113 also provides a gamification technique to improve the quality of the reports. For instance, checking the redundancy of the report, verifying the report, rate the quality of the report etc. In the end, the City113 application can help official authorities to open self-service channel to citizens, automate processes, and accelerate the quality and quantity of public service.

PART III: POSTERS ON WATER-FOOD-HEALTH NEXUS .....

Influences of Environmental Variables on Cyanobacterial Biomass and Microcystins Concentration in a Drinking-Water Supply Reservoir, Vietnam

THANH-LUU PHAM[1]*, THANH-SON DAO[2] , NGOC-DANG TRAN[3,4], UTSUMI MOTOO[5]

[1] Institute of Tropical Biology, Vietnam
[2]Ho Chi Minh City University of Technology, Vietnam
[3]Department of Health Care Policy and Management, Graduate School of Comprehensive Human Sciences, University of Tsukuba, Japan
[4]Department of Environmental Health, Faculty of Public Health, University of Medicine and Pharmacy, Vietnam
[5]Graduate School of Life and Environmental Sciences, University of Tsukuba, Japan
*Corresponding author: Thanh-Luu Pham

Abstract

Cyanobacterial blooms can decrease the environmental quality and be harmful to animals, and human health due to the toxic secondary metabolites of cyanobacteria, known as cyanotoxins. Microcystin (MCs), one of the most widespread and toxic cyanotoxins present in freshwater, can lead to skin irritation or liver and kidney damage as well as may initiate liver cancer. The concentration of MCs has been found to be positively correlated with cyanobacterial biomass, nitrogen and phosphorus availability in temperate lakes. However, in tropical water bodies, it remains unclear. Dau Tieng Reservoir, located about 85 km northwest of Ho Chi Minh City, has been supplying drinking water for millions of people in Ho Chi Minh City and nearby provinces. The reservoir falls into the eutrophic category with total phosphorus concentration of 25–100 µg/L and total nitrogen concentration of 600–1500 µg/L. Nutrient enrichment, especially phosphorus and nitrogen, has led to conditions that favor cyanobacterial growth in the reservoir. In this poster, we present the dynamics of cyanobacteria and MCs concentration as well as the influences of environmental variables on cyanobacterial proliferation and MC concentrations in the Dau Tieng Reservoir. Cyanobacterial biomass, MC content and environmental variables were monitored monthly from March 2012 to February 2013. Microcystin concentrations were quantified by high performance liquid chromatography (HPLC), whereas principle component analysis (PCA) and redundancy analysis (RDA) were applied to evaluate the influence of biotic and abiotic factors on cyanobacterial biomass and MCs concentration. The results showed that a total number of 3 orders, 16 genera and 42 cyanobacterial species were recorded from the reservoir. The MCs were found during the monitoring and the highest MCs concentrations of intracellular were in September and February,

2.5 and 2.13 µg/L, respectively, when cyanobacterial mass reached the maximum. The PCA and RDA showed that MCs concentrations were positively correlated with biomass of Chroococales, and total phosphorus was the primary abiotic factor influencing cyanobacterial biomass and MCs concentration in the Dau Tieng Reservoir. Microcystin concentrations in raw water were sometimes higher than the WHO guideline value of 1.0 µg/L for drinking water. Therefore, risks associated with exposure to MCs present in the raw water are a serious concern from the standpoint of human health. Hence, during periods of high MCs concentrations in the reservoir, local residents might suffer from hepatotoxic effects via their daily consumption of MC-contaminated drinking water. Management strategies of toxic cyanobacteria and their toxins as well as water quality from the Dau Tieng Reservoir are strongly recommended to reduce impacts on humans and ecosystems.

Keywords: cyanobacteria; microcystins; environmental influence; Dau Tieng Reservoir

Decolorization of Selected Synthetic Textile Dyes by Yeasts from Leaves and Fruit Peels

ANNA CHRISTINA R. NGO,[1,2] MARK KEVIN P. DEVANADERA,[1,2] GINA R. DEDELES*[1,2,3]

[1]The Graduate School, [2]Research Center for the Natural and Applied Sciences,[3]Department of Biological Sciences, College of Science, University of Santo Tomas, Philippines

*Corresponding author: Anna Christina R. Ngo, Laboratory of Pure and Applied Microbiology, Research Center for the Natural and Applied Sciences, Thomas Aquinas Research Complex, University of Santo Tomas, Philippines

Abstract

Textile dyes discharged into the water environment pose a big threat as they are poorly degradable and highly toxic to aquatic organisms and even to humans due to their complex organic composition and aromatic structures. In search for alternatives to physical and chemical treatments of these dyes, biodegradation ofsuch textile dyes by different microbes is emerging as an effective and promising approach.

This study aimed to use yeast co-cultures and consortia isolated from leaves and fruit peels and to determine their efficiency in decolorizing synthetic dyes alongside with their decolorization mechanism.

Yeasts were isolatedfrom leaves of rose, pineapple and mango, and fruit peels of pineapple which were collected from market waste. These substrates, even considered as wastes, could be a good source of microorganisms.These yeast isolateswere screened for potential decolorization of synthetic dyes at 25-50 µg/mL and were identified using API Identification kit.Decolorization parameters were optimized for the synergistic property and development of yeasts co-cultures and consortium. Possible decolorization reaction was assessed by cell immobilization using alginate beads, SDS-PAGE for partial characterization of extracellular enzymes involved in the study, and Fourier Transform Infrared Spectroscopy(FTIR) analysisfor detection of changes on the functional group of the synthetic dyes upon treatment.

Sixteenyeastswere isolated from leaves and fruit peels andonly 4 isolates showed high decolorization onfour synthetic dyes namely-Direct Pink B(DPB), Disperse Yellow 5G(DY5G), Direct Fast Orange S(DFOS), and Reactive Turquoise Blue G(RTBG). The optimum condition of decolorization for50 µg/mL of DPB were pH 9, 37°C for*Candida guilliermondii* Y011; pH 4 and 25°Cof *C. dubliniensis* Y014 for DY5G; pH 7 and 25°C of*C. guilliermondii* Y004 for DFOS; pH 4 and 35°C of*C. famata* Y003 for RTBG. The 4 isolates did not show any antagonistic activity that leads to the formation of co-cultures and consortium where the best co-cultures obtained 61% decolorization of DPB, 65% decolorization of DY5G, 41% decolorizationof DFOS and 50-51% decolorization of RTBG.

FTIR analysis showed that there were minimal changes on the structural components of RTBG and DFOS aftertreatment of certain organisms corresponding to its optimum decolorization. Meanwhile, for DPB and DY5G, the cells of yeasts appear colorless under the microscope which could be concluded as no adsorption that occurred between yeasts cells and the dyes, hence decolorization of the dyes still proceed even if the yeasts cells were immobilized. FTIR analyses confirmed that there were some changes in the functional groups of the dye structure which means that the yeasts may perform some other reactions other than adsorption.Other factors such as extracellular enzymes may contribute to the

decolorization of dyes and SDS-PAGE analysis confirmed the presence of these enzymes that has been released when the yeast isolates were immobilized.
Theseyeast isolates or their enzymes could be used for possible treatment in wastewater systems where there are secretions of synthetic dyes or for wastewater treatment in industries using synthetic organic dyes.

Physiological Elucidation, Genetic Architecture and Metabolic Landscape of Tolerance to Flooding during Germination in Rice

FREDERICKSON D. ENTILA, MYRISH A. PACLEB, EVANGELINA S. ELLA, ABDELBAGI M. ISMAIL

Plant Physiology, Crop and Environmental Sciences Division, International Rice Research Institute, Laguna

Abstract

As the arable land is definite, weather is unfavorably changing, and natural resources are continually deteriorating, with the demands of dramatically bloating population, food scarcity is imminent. Furthermore, food production suffers from increasing urbanization and negative impact of climate change through loss of land, its productivity, and decline in labor forces for agricultural purposes. These emerging problems substantiate the urgent need to enhance adaptation of agriculture crops through intensification of cultivation, sustainable food systems, and augmented genetic gains. Intensification of rice production through direct seeded rice (DSR) has been progressively practiced but wide implementation is hampered due to limited germination and poor crop establishment of existing varieties under flooded conditions. Identifying donors of tolerance to anaerobic germination (AG) will facilitate varietal improvement for DSR and will result to enhanced and ecological agriculture. The Rice Diversity Panel 1 (343 accessions) consisting of 5 subpopulations was screened for AG tolerance. Analyses revealed that most of the tolerant varieties are japonicas, with some indicas and admixed. Tolerance is attributed to fast shoot growth but substantial root extension under flooding. Relating genotypic and phenotypic components showed that the tolerance is controlled differently across subpopulations suggestive of existence of various tolerance mechanisms. Genome wide mapping revealed that most traits had association peaks at chromosomes 1, 4, 5, 6, 9, 11, and 12: some co-localized with known QTLs and others were novel. Candidate gene network analyses implied that most genes are involved in metabolic processes and functions as catalyst and in binding. Upon metabolite profiling, 1221 metabolites were detected, 102 of which were significantly differentially expressed: 68 up-regulated and 34 down-regulated. Apparently, populations were being developed from identified donors to facilitate breeding platform towards enhanced and sustainable rice production. Moreover, metabolites will be profiled through the germination course to capture metabolic adjustments under flooding stress.

Keywords: direct seeding, anaerobic germination, genome-wide association mapping, metabolite profiling

Comparison of Seasonal Total Water Storage Variations from Grace with Groundwater Levels, Stream Flow and Soil Moisture in Southern Laos

SINXAY VONGPHACHANH

School of Civil and Environmental Engineering, Faculty of Engineering and Information Technology, University of Technology, Australia

Abstract

The Gravity Recovery and Climate Experiment (GRACE) gravity satellite program was launched jointly by the National Aeronautics and Space Administration (NASA) of the United States and the German Aerospace Centre (DLR) in March 2002 to measure changes in the earth's gravity field (Tapley et al. 2004). The movement of mass in surface water, soil water and ground water causes these changes. GRACE measurements are processed mathematically to extract estimates of total water storage (TWS) expressed as equivalent water height (EWH) with a spatial resolution of about 200,000 km^2. In 2013, a new website for GRACE was developed by The Australian National University (ANU) using the spherical harmonic fields of the French Groupe de Rechercheen GéodesieSpatiale (GRGS). This website provides a Data Visualisation Tool (DVT) by which users can estimate the EWH in a user specified region (polygon) or point with a spatial resolution of about 62,500 km^2. GRACE data has been widely utilised to determine the variations of groundwater storage in many countries. However, this has not yet been implemented in Laos. Therefore, the main objective of this study is to investigate the feasibility of applying GRACE satellite data to estimate total groundwater storage in Southern Laos by comparing time series data of GRACE-derived TWS with groundwater levels, streamflow observations and soil moisture. A basin scale of about 25,000 km^2 was utilised to investigate the GRACE-derived total water storage correlation with in situ groundwater levels and measured streamflow in southern Laos, one of the main target areas for agricultural development. The total groundwater availability in this area is currently not yet known exactly. This study presents the first direct comparison of total water storage derived from GRACE satellite missionwith in situ hydrological monitoring. Monthly time series of soil moisture derived from the Global Land Data Assimilation System (GLDAS) and TWS drawn from GRACE between November 1, 2011 and April 30, 2013 are compared with groundwater levels from a piezometer network in Sukhuma district and Mekong River flow at Pakse hydro-meteorological station in Champasak Province, Southern Laos. Moreover, seasonal soil moisture maps derived from GLDAS are also compared with surface soil, land use and vegetation cover maps. The results illustrate that the GRACE-derived TWS agrees with the on-site groundwater table and streamflow measurements. In addition, comparison between soil moisture derived from GLDAS and measured groundwater table elevation in Sukhuma district and flow at Pakse station demonstrates similar seasonal fluctuations. The comparison between seasonal soil moisture maps and surface soil and vegetation maps shows that the highest volumes of soil moisture are usually found in Shrub land areas underlain by sandy loam soils. These preliminary results could be useful for estimating total groundwater storage variations and availability from GRACE data for Sukhuma district and Southern Laos in the future.

Keywords: GRACE, GLDAS, Seasonal variations, Total Water Storage, Soil moisture, Groundwater levels, Southern Laos

Risk Assessment of Salmonella and Vibrio Cholerae Pacini in Pacific White Shrimp (*Litopenaeus vannamei Boone*) in Southern Philippines

MARIA ELENA N. TANABE[1], IDA F. DALMACIO[2] AND AGNES F. ZAMORA[2]

[1]University of Southern Mindanao,
[2]University of the Philippines Los Baños
Corresponding author: Maria Elena N. Tanabe, University of Southern Mindanao, Philippines

Abstract

A risk assessment of Salmonella and Vibrio cholerae in Pacific white shrimps consumed in Southern Philippines was conducted. This study estimated the prevalence and concentration of the total Salmonella and V. cholerae in Pacific white shrimps at harvest and retail stages. Consumption of Pacific white shrimps and cooking efficiency were studied using interviews and onsite observation of consumers. A beta-Poisson dose-response model was used to estimate probability of illness. Microbial and consumption data were analyzed by developing a stochastic model and the simulation gave a mean number of times a person would get ill with V. cholerae by consuming Pacific white shrimps at 5.03×10^{-8} . Quantitative risk assessment was not performed for Salmonella in Pacific white shrimp because no Salmonella was detected during the analysis. Sensitivity analysis demonstrated that the initial population of active V. cholerae in shrimp samples, fraction of people who did not cook the shrimps properly, and the amount of shrimp consumed were the primary factors in increasing risk. This study serves as an example of how a microbiological risk assessment with limited data collection leads to valuable local insight on shrimps' safe consumption.

Biocatalytic Formation of Norisoprenoids as Natural Flavor from Carotenoids as Natural Colorants in Pandan Leaves (*Pandanus Amaryllifolius*)

ANDRIATI NINGRUM[1], NHUT NGUYEN MINH[2], MATTHIAS SCHREINER[2]

[1]Department of Food Science and Agricultural Technology, Faculty of Agricultural Technology, Gadjah Mada University, Indonesia
[2]Institute of Food Science, BOKU University of Natural Resources and Life Sciences, Austria

Abstract

Degradation products of carotenoids, known as norisoprenoids, are aroma impact compounds in several plants. This oxidative enzymatic degradation is mainly caused by endogenous carotenoid cleavage enzymes (CCDs). Pandan wangi is a common name of a shrub called Pandanus amaryllifolius. This plant is often used as a flavoring component. The general objective of this research was to determine the enzymatic carotenoid cleavage activities of pandan leaf extracts by using two different carotenoid substrates (β-carotene and β-apo-8'-carotenal), to determine the influence of temperature and pH to the enzymatic activities and to determine the enzymatic reaction products by using HS-SPME GC-MS. Crude enzymes from pandan leaves showed higher activity on β-carotene than on β-apo-8'-carotenal. The optimum temperature of crude enzymes was found at 70oC while the optimum pH was found at 6. β-ionone was found to be the major volatile reaction product from the incubations of both carotenoid substrates (β-carotene and β-apo-8'-carotenal).

Edible Film Packaging as Nutraceutical Delivery Vehicle to Improve Human Health

PHONG T. HUYNH[1] ; PAUL TAKHISTOV[2]

[1] Ho Chi Minh City University of Technology, Vietnam
[2] Rutgers- The State University of New Jersey, USA

Abstract

It has been claimed that antioxidant compounds widely found in fruits and vegetables bring health benefits. However, their low solubility decreases their adsorption rate in living system leading to the low bioavailability. Among several techniques being explored to increase the bioavailability of this compound, utilization of nanoparticles is considered a promising method to improve the solubility of hydrophobic compounds. Because nanoparticles provide greater surface area as well as increased dissolution pressure.

Traditional methods for preparing nanosuspensions include jet milling and pearl milling where the macro particles are broken down by mechanical stress under high pressure. These techniques are high energy consumption and generate nanoparticles with broad size distribution. On the other hand, Emulsion-based method is a promising practice to generate nanoparticles. This process not only requires less energy than the traditional ones but also generates nanosuspension with small particle size distribution.

The introduction of nutraceutical at nano size into food matrix faces several challenges since food is complex system. Nanoparticle may interact with other components in food leading to the change in texture, or causing aggregation of the particles. Among food products, edible filmis a good candidate for encapsulating and delivering nutraceuticals.

The objective of my research is to utilize edible film as a vehicle to deliver nano-size nutraceuticals to improve human health. Beta-carotene has been selected as a nutraceutical model. The emulsion-based method, where “Generally Recognized as Safe” substances have been used, is applied for preparing the beta-carotene nanosuspension. Impact of several factors to the size and particle size distribution is investigated. Kolmogorov dissipation theory is used to integrate the breakup of emulsion droplet. A mathematical model is built to predict the particle size of nano suspension. Hydroxypropyl methyl cellulose is chosen for preparing edible film. The polymer-surfactant interaction in aqueous media and during film formation is investigated. Our results show that the emulsion droplets break under inertial regime; lecithin is good surfactant for preparing nanosuspension. The results also show the influences of polymer – surfactant interaction to the drying kinetic of polymer solution and impact of beta-carotene nanoparticle to the moisture adsorption of the film.

Transformation Products of Sulfamethoxazole in Aqueous Samples after 60co Gamma Irraduation

NAM LE-DUY [1,2], THANG NGO-MANH [1]

[1] Faculty of Chemical Engineering, University of Technology – Vietnam National University-HCM, Vietnam

[2] Forensic Chemistry Agency, Dong-Nai Province, Vietnam

Abstract

In Vietnam, most antibiotics for human healthcare are still available without prescriptions. Moreover, other antibiotics are widely used in different agricultural activities. Therefore, some residues in surface water in Vietnam were found at much higher levels than in Japan and other countries, especially residues of sulfamethoxazole (Shimizu et al 2013). Antibiotic residues in aquifer systems are considered as potential threats to the public health and their treatment attract attention worldwide. E.g. treatment of sulfamethoxazole in water using visible and UV-treatments as well as using various advanced oxidation processes was reported (Trovo et al 2009, Kim et al 2009, Canonica et al 2008, Beltran et al 2008, Baeza and Knappe. 2011, Katsoyiannis et al 2011, Lekkerkerker-Teunissen et al 2012). Recently, gamma irradiation was applied to aqueous samples containing sulfamethoxazole (100 & 1000 µM) under different conditions, promoting either oxidative or reductive reactions (Sagi et al 2014). Sulfamethoxazole removal and its complete removal were achieved at doses 5 kGy and higher, respectively. A LC/MS-MS method revealed many peaks of sulfamethoxazole degradation products, but only 3 were discussed in details.

Motivating by the governmental decisions to build up atomic power plants in the near future, application of gamma irradiation to various industrial spheres including water treatment and –reuse has attracted our attention (Ngo et al 2010, Le et al 2014).

This poster describes our recent results obtained during a MEng thesis. Transformation of sulfamethoxazole dissolved in aqueous samples after ^{60}Co gamma irradiation was investigated as functions of initial sample compositions: pH_0 (2 ÷ 11), concentrations $[SMX]_0$, (20 µM ÷ 140 µM) and $[H_2O_2]_0$ (0.1 mM ÷ 0.5 M), and absorbed doses D (0.3 kGy ÷ 5.0 kGy). Transformation products were evaluated by means of HPLC/UV and LC-TOF-MS methods. 5 products were registered on the HPLC/UV and LC-TOF-MS chromatograms. Compared to sulfamethoxazole, all these 5 products appeared to have higher polarity and molar mass (m/z = 270, 271, 286, 288, 304). Among them, the one with m/z = 270 was the most abundant. With increasing the absorbed doses D up to about 1.0 kGy, the abundances of all these 5 products increased. Further increasing the absorbed doses resulted in decreasing these abundances, reaching their nondetectable states at D = 5.0 kGy. Based on the m/z values of detected ions, the corresponding reaction schemes are proposed, discussed and compared to the literature data.

Keywords: Sulfamethoxazole, gamma irradiation, transformation products, transformation mechanism.

References

Akiko Shimizu, Hideshige Takada, Tatsuya Koike, Ayako Takeshita, Mahua Saha, Rinawati, Norihide Nakada, Ayako Murata, Tokuma Suzuki, Satoru Suzuki, Nguyen H. Chiem, Bui Cach Tuyen, Pham Hung Viet, Maria Auxilia Siringan, Charita Kwan, Mohamad P. Zakaria, Alissara Reungsang. 2013. "Ubiquitous occurrence of sulfonamides in tropical Asian waters". *Science of the Total Environment*. 452-453: 108-115.

Alam G. Trovó, Raquel F.P. Nogueira, Ana Agüera, Carla Sirtori, Amadeo R. Fernández-Alba. 2009. "Photodegradation of sulfamethoxazole in various aqueous media: Persistence, toxicity and photoproducts assessment". *Chemosphere*. 77(10): 1292-1298.

Alam G. Trovó, Raquel F.P. Nogueira, Ana Agüera, Amadeo R. Fernandez-Alba, Carla Sirtori, Sixto Malato. 2009. "Degradation of sulfamethoxazole in water by solar photo-Fenton. Chemical and toxicological evaluation". *Water Research*. 43(16): 3922-3931.

Carolina Baeza, Detlef R.U. Knappe. 2011. "Transformation kinetics of biochemically active compounds in low-pressure UV Photolysis and UV/H_2O_2 advanced oxidation processes, *Water Research*. 45(15): 4531-4543.

Fernando J. Beltrán, Almudena Aguinaco, Juan F. García-Araya, Ana Oropesa. 2008. "Ozone and photocatalytic processes to remove the antibiotic sulfamethoxazole from water". *Water Research*. 42(14): 3799-3808.

Gyuri Sági, Tamás Csay, György Pátzay, Emil Csonka, László Wojnárovits & Erzsébet Takács. 2014. "Oxidative and reductive degradation of sulfamethoxazole in aqueous solutions: decomposition efficiency and toxicity assessment". *J Radioanal Nucl Chem*. 301:475-482.

Ilho Kim, Naoyuki Yamashita, Hiroaki Tanaka. 2009. "Photodegradation of pharmaceuticals and personal care products during UV and UV/H_2O_2 treatments". *Chemosphere*. 77(4): 518-525.

Ioannis A. Katsoyiannis, Silvio Canonica, Urs von Gunten. 2011. "Efficiency and energy requirements for the transformation of organic micropollutants by O_3, O_3/H_2O_2 and UV/H_2O_2". *Water Research*. 45(13): 3811-3822.

Karin Lekkerkerker-Teunissen, Mark J. Benotti, Shane A. Snyder, Hans C. van Dijk. 2012. "Transformation of atrazine, carbamazepine, diclofenac and sulfamethoxazole by low and medium pressure UV and UV/H_2O_2 treatment". *Separation and Purification Technology*. 96: 33-43.

Le Thi Thuy Trang, Tran Thi Tuu, Le Thi Thuy, Hoang Minh Nam, Ngo Manh Thang. 2014. "Transformation of 2-naphthol in water by gamma-, UVC- and simulated solar irradiation". *J Sci Technol (in Vietnamese)*. 52(4B): 316-323.

Silvio Canonica, Laurence Meunier, Urs von Gunten. 2008. "Phototransformation of selected pharmaceuticals during UV treatment of drinking water". *Water Research*. 42(1–2): 121-128.

Thang. M. Ngo, Nam M. Hoang, Tram T. M. Tran. 2010. "Radiolysis of 1-naphthol in aqueous solutions", *J Radioanal Nucl Chem.* 286: 287–293.

Effects of Tocotrienol Supplementation on Biomechanical Strength of Tibia in Rats with Testosterone Deficiency

CHIN KOK-YONG, DHIVAKARAN GENGATHARAN, FADLIN SAKINA MOHD NASRU, REHAN AMALIA KHAIRUSSAM, SHERLYN LAI HUI ERN, SITI AINA WAHIDAH AMINUDDIN, IMA NIRWANA SOELAIMAN

Department of Pharmacology, Universiti Kebangsaan Malaysia Medical Centre, Malaysia
Corresponding author: Chin Kok-Yong, Department of Pharmacology, Universiti Kebangsaan Malaysia Medical Centre, Malaysia.

Abstract

Background/Aim

Osteoporosis is characterized by the deterioration of bone mass and microarchitecture, leading to reduced bone strength and ultimately fractures. Testosterone deficiency is the leading cause of osteoporosis in men. Bone-strengthening agents available in the market, apart from calcium and vitamin D, are limited. Previous studies highlighted that tocotrienol, an isoform of vitamin E, exhibited osteoporosis-preventive properties in animal models of osteoporosis. However, studies on the effects of tocotrienol on bone in osteoporosis model induced by testosterone deficiency are limited. This study aimed to determine the effect of tocotrienol on bone biomechanical strength in a rat model of osteoporosis due to testosterone deficiency.

Methods

Thirty Sprague-Dawley male rats aged three months old were randomized into 5 groups, i.e. baseline (BL), sham (SH), orchidectomized (ORX), tocotrienol-treated (T3) and testosterone-treated group (TT). The BL group was sacrificed upon received. The ORX, T3 and TT-treated group were orchidectomized. Tocotrienol (60 mg/kg body weight) derived from annatto (composition: 90% delta-tocotrienol and 10% gamma-tocotrienol) was given to the T3 group orally once daily while the other groups were given olive oil as the vehicle. The TT group received intramuscular injection of testosterone enanthate (7mg/kg) once weekly while the other groups received peanut oil as the vehicle. After eight weeks of treatment, the rats were sacrificed and their right tibias were harvested for biomechanical strength test. A modified three point-bending test was adopted to test the biomechanical strength at the distal tibia using the Instron device (MA, USA). The serum of the rats was collected for the determination of serum calcium and phosphate level using enzyme-linked immunosorbent assay. Comparison of mean in variable of interest among the groups was performed using one-way analysis of variance.

Results

Strain and elasticity of the tibia were significantly higher in the T3 and TT group compared to ORX group ($p<0.05$). Load and stress between the treated groups and the ORX did not differ significantly ($p>0.05$). In comparison with the ORX group, serum calcium level was significantly lower in the TT group ($p>0.05$), and marginally lower in the T3 group ($p=0.058$). Serum phosphate level was not significant different between the treated groups and the ORX group ($p>0.05$)

Conclusion

Tocotrienol can improve certain biomechanical properties of the bone. It has the potential to be developed as a bone-strengthening agent in men suffering from osteoporosis due to testosterone deficiency. Its efficacy is comparable to testosterone replacement therapy. This study provides justification to conduct a clinical trial to validate the effects of tocotrienol on bone in osteoporotic patients.

Keywords: Bone; Calcium; Osteoporosis; Strength; Vitamin E.

Effect of Light and Hydrodynamics to Producing Spirulina Biomass in ThePhotobioreactor System Designed in Viet Nam

VU VAN PHUOC[1], MAI THI HONG HANH[2], PHAM VIET HUNG[1,2]

[1.] Center of Research and Technology Transfer, Viet Nam Technical Scientific Application JSC, Vietnam
[2.] Clinical Laboratory, Viet Nam National Children Hospital, Vietnam
Corresponding author: Pham Viet Hung

Abtract

The cyanobacteria Spirulina is known as the high protein and vitamins source. Recently, they are interested and applied in some important fields such as pharmaceuticals, functional food and cosmetic industries. The Spirulina cultured is affected by many different factors such as light intensity, hydrodynamics, and contamination of other microbes. In this study, we used the photo-bioreactors system (PBR), with investigation of optimal light density and hydrodynamics for Spirulina biomass harvesting. As brief description, the system was made by unleaded glass tube with 1260mm and 660mm in length and 32mm in diameter, with fluorescent light and ceramic stirrer. Total power consuming was 400W, length of cycling pipes was 80m, temperature was maintained 23-24^{0}C. Results: after five days of culture, the maximum biomass was 68.3 mg/l, with optimal condition was 850 lux of light intensity, 24/24h of lighting time, 0.2 – 0.3 m/s of velocity, and hydrodynamics Reynolds number ranged1 – 2x10^4. Conclusion: This domestic model of closed, cycling PBR would be applied for producing Spirulina biomass in Viet Nam, with advantages as simple design and low cost. We show here optimal light density and hydrodynamics for this model, which might be used for other similar system.

PART IV: POSTER ON INFORMATION AND COMMUNICATION TECHNOLOGIES……..

Community-based ICT Infrastructure for Local Content Aggregation

R.C. DAGUIL, F.M. DAGUIL1, G.A. DALAORAO

ICT Center, Caraga State University, Philippines

Abstract

Information is a basic commodity in today's digital world. With the proliferation of mobile devices, local content can easily be generated at their fingertips even in rural areas. This content may include products, goods, services, announcements, issues and concerns, and other localize information contextual to the community. However, with the computing devices in their hand, target market audience can be extended using appropriate technology and community-based ICT infrastructure. This paper presents a community-based ICT infrastructure to aggregate local contents using push technology in mobile devices. The technology provides unobtrusive wireless connectivity utilizing Bluetooth, WIFI, Internet over GSM and SMS. The aggregated contents are stored in a centralized location equipped with lightweight server, access point and display devices that are redundantly powered by solar energy for continuous operations. Exchange protocol between communities is employed using REST-based web services along with security mechanism. Local contents are posted and displayed in a context-sensitive manner where local products, goods and services are group and classified for localization.

Keywords: rural informatics, content aggregation, mobile push technology