PROCEEDING

INTERNATIONAL SEMINAR ON SCIENCE AND TECHNOLOGY 2014

October 23, 2014

Tegalboto Campus, University of Jember
Jember, Indonesia

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Proceeding of The International Seminar on Science & Technology 2014 (**ISOSTECH '14**)

The editing of this proceeding has been carried out by **Siswoyo & B. Kuswandi** with assisted by the Scientific Committee of ISOSTECH'14.

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Foreword by Organising Committee

Assalamu'alaikum Wr. Wb.

Distinguished guests and delegates

On behalf of the organizing committee, I am deeply grateful to your present in the International Seminar on Science & Technology 2014 (**ISOSTECH '14**) that already held in Universitas Jember, Jember Indonesia on thirsday, 23 October 2014.

The **ISOSTECH '14** is jointly seminar between University of Jember (UNEJ), Indonesia and Universiti Sains Islam Malalaysia (USIM), it was arranged with substantive elements such as seminar pertaining to current advance on science and technology together with posters.

The seminar was provide an excellent platform for knowledge exchange between the academicians, researchers, scientists and engineers working in areas of mathematic and basic sciences, agricultural and food Technology, health sciences and enggineering as well as information technology. In addition, it provides an opportunity for the participants from Indonesia, Malaysia and Philiphine to share research findings, to establish networking and to encourage academic and student exchange and other participation in this exciting seminar.

We also would like to express our deep appreciation to the all organising committee members and steering committee, especially Dr. Zulfikar, on behalf of Rector, as Vice Rector of UNEJ who officially opens this seminar. Last but not least our appreciation to all participants especially delegate from USIM, IIU Malaysia and San Carlos University, Philiphines. We convey our great gratitude for your scientific speech and contribution. We do hope that all these research results are useful for further research progress and development in these fields.

Enjoy the conference proceeding and hope it will give inpiration on your research projects.

Wassalamu'alaikum Wr. Wb.

Mrs. L. Wulandary
Chairperson
University of Jember

Preface

The first International Seminar On Science & Technology 2014 (**ISOSTECH '14**), took place in University of Jember, Jember East Java Indonesia on 23 October 2014. This first seminar series is focused on all aspects related to recent advance in science and technology.

This proceeding contains papers that have been presented at **ISOSTECH '14** as plenary lectures, invited, oral and poster presentations. About 100 participants attended the conference, with 4 plenary lectures, 35 oral and 24 poster presentations. The proceeding of **ISOSTECH '14** has been published in electronic form as *.pdf file for simple and easy publication and to avoid heavy book of proceeding. We hope that this publication can be easily read, handled and transferred to other form. Furthermore, this paperless proceeding can be fruitful for all participants of the conference.

My sincerely thanks go to all the members of Scientific Committee for their valuable help in the review of the submitted papers, and also to the authors for their collaborative attitude. A special mention must go to **Mrs. L Wulandary**, our Conference Chairperson, who has put in a terrific amount of effort not only in general conference matter but also in the assembly of the papers for this proceeding. Finally, I congratulate the authors of all papers for producing the new and novel idea for research on mathematic and basic sciences, agricultural and food Technology, health sciences and enggineering as well as information technology.

Jember, October 2014

Siswoyo & B. Kuswandi
Editors

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POSTER SESSION

Environmental Impact Assessment (EIA) and The Needs of Socioeconomic Impact Assessment (SIA) on The Vulnerability of Human Population: A Case Study of Landfill Project in Kangar, Perlis, Malaysia

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Abstract **-** Environmental Impact Assessment (EIA) is an assessment of possible positive or negative impact that the proposed project may have on the environment. The environment consists of Physical Environment, Biological Environment, and Human Environment. Socioeconomic Impact Assessment is valuable in presenting a picture of socioeconomic condition in a community and how they have reacted to the new proposed project involving their surroundings. Human Vulnerability is the capacity to be wounded by socioeconomic and ecological changes. Human population that are exposed to substantial stress, are sensitive to change; and the lack of resiliency are vulnerable to the declining quality of life because they are unable to respond to socioeconomic, institutional and environmental changes. The paper aims to analyze the impact categories on the village people resided in Kg Kubang Tiga and Felda Rimba Mas, in Kangar, Perlis, due to the development of the landfill project, using the macroeconomic model and its implications on the environmental sustainability.

Keywords: EIA, SIA, Human Vulnerability, Landfill

1. Introduction

EIA is being used as a decision aiding tool rather than decision making tool with regards to the assessment in environment. It addresses the direct on-site effects alone. However, development cause a multitude of indirect impacts through consumption of goods and services, production of building materials and machinery, additional land-use, mining resources, etc. It is an order of magnitude higher than the direct effects assessed by the EIA, such as proposed airports, shipyards, and others, should be taken into consideration during decision making process.

The scope and method available to include firstly, the Industrial Products, which consider the technological activities used for various stages of the product-till the disposal of the products. Secondly, the Genetically Modified Plants, which consider the specific methods available to perform EIAs of genetically modified plants. Some of these methods are GMP-RAM, INOVA, etc. Thirdly, the Fuzzy Arithmatic, which consider an EIA method need specific parameters and variables to be measured to estimate values of impact indicators, and many of the environment impact properties cannot be measured on a scale value, for example landscape quality, lifestyle quality, social acceptance, which is very subjective, will use Fuzzy logic approach. Finally, an audit, where EIA audit evaluates the performance of an EIA by comparing actual impacts to those that were predicted, the objective is to make future EIAs more valid and effective. They are the scientific, in order to check the accuracy of predictions and explain error; and the management, in order to assess the success of mitigation in reducing impacts.

Types of EIA

There are 2 types of EIA procedures used by the Department of Environment (DOE), namely the Preliminary EIA (PEIA) and the Detailed EIA (DEIA). PEIA procedure is applicable to all prescribe activities except those that are subject to DEIA. The DEIA procedure is required for projects that are deemed to have major impacts on the environment. Activities that require DEIA include iron and steel industry, pulp and paper mill, cement plant, construction of coal fired power plant, construction of dams for water supply & hydroelectric schemes, land reclamation, incineration plant (scheduled wastes and solid wastes), construction of municipal solid waste landfill facility (including municipal solid waste transfer station). Other DEIA to include project involving land clearing where 50% of the area or more having slopes exceeding 25 degrees (except quarry); logging covering an area exceeding 500 hectares or more; development of tourist or recreational facilities on islands in surrounding waters which are gazette as national marine parks; construction of recovery plant (off-site) for lead-acid battery wastes; scheduled waste s recovery or treatment facility generating significant amount of wastewater located upstream of public water supply intake; non-ferrous-primary smelting.

The Director General may require other projects to undergo the DEIA process if she/he has reasons to believe the proposed project has major and significant environmental impacts and is of public interest.

Identifying Environmentally Sensitive Areas (ESAs)

- EIA report should clearly identify and map all ESAs within a 3 to 5 km radius from the project site (Zone of Influence-ZOI).

- ESAs can be defined as areas that are sensitive to development and need to conserve for its heritage and life support values and minimize the hazard risks due to land use change.
- The activities which should be considered as ESAs are all types of forests, wetlands, wildlife reserves, water catchment areas (including groundwater abstraction), areas of scenic beauty, highlands and areas of steep slopes, geohazards, floodplains, and historical and/or archeological sites.

Public Participation

The methods for public participation include public briefing or/and public dialogues;focus group dialogues; project information kits; questionnaires surveys; and one-to-one interviews.

Areas of Specialization

Areas of Specialization in EIA to include Forestry; Health Impact Assessment; Ecological Studies; Quantitative Risk Assessment; Fisheries; Chemical Processes; Hydrology; Water Quality; Industrial Processes (other than chemical processes); Noise and Vibration; Land-use Planning; Air Quality and Odour; Mining; Landscape and Visual; Quarrying; Geology and Soil; Scheduled Waste Management; Geotechnical Study; Solid Waste Management; Hydrological Study; General Environmental Management; Archeology; History; Traffic Impact Assessment; Social Impact Assessment/ Socioeconomics Impact Assessment/Socioeconomics; Demographic Impact Assessment; and Economic Evaluation.

The Objectives

a. To describe a preliminary assessment of the existing socioeconomic environment conditions which are likely to be affected by the proposed project on the vulnerable segment of the human population.
b. To gather preliminary local public opinions especially the vulnerable people and their views on the implementation of the proposed project. Hence, the study is expected to solicit the degree of acceptance and opposition, including the condition set by the local public on the implementation of the proposed project.

The Theoretical Framework

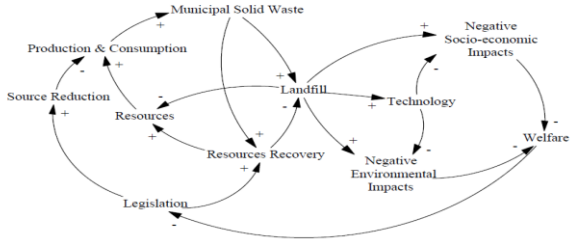

Figure1: Causal Loop Diagram

Figure 1 shows the causal loop between landfill and other related activities. Human activities, rubbish from industries, private commercial buildings and stratified properties, production and consumption are inevitable where solid waste is the by-product of these activities. When the production and consumption increase, this will increase the municipal solid waste. Yet rubbish is something we must deal with, and where there is demand, there is supply. Anyone with a lorry can become a waste removal contractor as there are no special licence required for the transport of waste under the law, except for toxic waste. The magnitude of change in municipal solid waste will be influenced by different factors such as population, per capital income, industrialisation, urbanisation, living standards, seasonal occasions, etc. When the municipal solid waste accumulates, the local authorities must find a way to dispose it. The easiest, simplest and most probably the most thrifty way to dispose municipal solid waste is to dump it at crude dumping landfills. Dumping all municipal solid waste in landfill without any recovery will cause the natural resources deplete and will in a way have negative effects on usage, productions and ultimately lower consumption which might in turn lower the living standard. On the other hand, haphazard dumping municipal solid waste at the landfill will lead to various negative environmental and socio-economic impacts such as ground water and surface water pollution, air pollution, health hazard and so on. These impacts will have severe chain reactions which will cause the degeneration of public welfare. In realising that crude dumping is not the only solution of handling the increasing municipal solid waste problems, the local authorities or the landfill managers will bring in new landfill technologies in order to prevent or at least to minimise the negative impacts that crude dumping landfills will lead to. Lining system, leachate collection and treatment and gas venting facilities will be installed at the landfill. By doing so, the socio-economic and environmental impacts will be reduced and thus indirectly public welfare will increase as compared to the situation where crude dumping is practised. Even with the new installed technologies, problems still exist. As long as the production and consumption activities are going on, the municipal solid waste will increase and the public welfare will still be threatened. They will, via various channels, complain to the authorities and this will lead to legislation to tackle the municipal solid waste problems. A legislation that brings to source reduction and resources recovery can reduce the municipal solid waste being sent to landfill in two different ways. Firstly, the source reduction policy will reduce the production and consumption which will reduce the municipal solid waste from being generated and of course the municipal solid waste that will be sent to landfill for disposal. The second solution is resources recovery where the municipal solid waste will first be sent for pre-treatment such as recycling, composting and incineration in order to recover as much resources as possible. These recovered resources will be added up to the resources availability to produce more goods. So the actual municipal solid waste that is sent to landfill for final disposal will be significantly reduced.

The Impacts of Landfill on Socioeconomics.

Landfill will not only lead the environmental destruction but also create other socio-economic problems if it is not conducted in a proper and well-

managed manner. The immediate perception about landfill is the unaesthetic view where waste is dispersed by wind and animals. Closely related to this is the odour that lingers out from the landfill site. Residents who stay at the downwind will always suffer from the odour and to the worst case their health might be affected by the pollutants. Other than that, dumpsites which are without daily cover will also attract scavengers to look for income at the site, this will not only create nuisance to the landfill staff but to the neighbourhood as well. The impacts of the landfill will indirectly lower the property value of the nearby areas. Despite of the negative impacts, with proper design and operation maintenance, landfill can reclaim sub-marginal land which otherwise would be unsuitable for any kind of land-use purposes. Sanitary landfill can be developed into a recreation area for the residents.

Activities of Scavengers

The recycling in the developing countries is largely depended on scavengers in recovering materials. This is done by scavengers at the dumpsites looking for recyclables for exchange of money. These scavengers constitute the poor and vulnerable segments of the population who face multiple problems and are perceived as a nuisance not only to the landfill operation but also to the society [1]. Sometimes scavengers could hinder the effective flow of land filling operation and to some extend could pose danger to environment by setting fire at the landfill or dumpsite [2]. Compared to others, scavengers are most exposed to health hazard posed by the landfill. They are normally not equipped with any safety boot, glove or mask when they are scavenging at the landfill site. These scavengers, mostly young and low income people are vulnerable to waste related diseases such as AIDS infection due to contacting with the infected medical instruments that disposed at the dumpsite [3].Though scavenging could cause some nuisances to the landfill staff and the neighbourhood, an attempt to abolish scavenging of waste would lead to mechanical separation and the cost of the recyclables would be not favourable for recycling [2]. And as for landfill without sorting facilities, the total elimination of scavenging could lead to the loss of the materials which could otherwise be recovered.

Value of Property

Landfill siting will bring an adverse effect on land values. There is a norm that public will put a lower value for property near the landfill. This is because landfill is considered as one of the environmental dis-amenities which will reduce the public welfare such as by causing health hazards and un-aesthetic view. Thus, the existence of landfills will greatly influence purchasers' buying decision and their bargaining power. According to a research in Ohio, United States, the property values tend to have negative correlation with landfills, assuming other environmental dis-amenities remain unchanged [4]. The external costs of the landfill that reflect on the lower property value might lead to unequal distribution of environmental quality where the disadvantaged socio-economic group will migrate to the areas near landfill site because of the lower housing price [4]. The property value impact of landfill will not only lower the housing price but also cause unequal distribution of environmental goods among different socio-economic group.

Social Considerations

Social consideration is another important element in determining the feasibility of sanitary landfill. Generally, people are reluctant to have landfill to be situated near their neighbourhood even though the landfill is equipped with the leachate collection and treatment facilities, gas collection facility, daily cover, large buffer zone, etc. People are not convinced that the sanitary landfill will not pose any threats to them. Basically, as long as the disposal site is not located in their neighbourhood, there will not be many objections from the people [5]. According [4], the people who live nearby to landfill site are generally perceived as lower socioeconomic group. Property value in this neighbourhood is relatively low as compared to other areas. Furthermore, building a sanitary landfill would consume a large land area. This will compete with other land-use purposes such as road construction, dam construction, housing scheme and other public services and utilities. Due to the increasing land scarcity, one development project has to forgo for another. If landfill has been chosen for the purpose, the opportunity for other development projects will have to be sacrificed. On the other hand, if the sanitary landfill is well designed, managed and monitored, it can be converted into recreation park and garden, golf course, car parking areas, etc [6].

2. The Methodology

Information will be elucidated from three main sources. Firstly, gathering and compiling information from secondary sources i.e. published and unpublished reports on existing socioeconomic environment conditions on the proposed site. Secondly, a preliminary assessment and verification on the existing information from the secondary sources will also be carried out. Any data gaps from the secondary sources will be compensated through liaison with relevant authorities. Finally, a site survey is expected to be significance to enable experts to gather the awareness and perceptions and identify community attitudes and perceptions on the proposed project. In order to identify the direct and indirect impacts, the selected sample size includes respondents within 5km radius from the proposed project site. A stratified random sampling technique is used to facilitate the questionnaire survey of the socioeconomic status. This information provides the necessary guidelines for gauging socio-economic impact and appropriate mitigation.

3. The Vulnerability Based Approach Model

Figure 2 shows the impacts of macroeconomic policies using a vulnerability-based approach framework to include review of proposed project, vulnerabilities filter, mapping vulnerabilities, assessing the impacts and developing recommendations. The purpose of reviewing the proposed project is to determine whether the assessment is necessary and

also to determine which economic and environmental sectors need to be considered by the assessment. The purpose of vulnerability filter is to determine which environmentally vulnerable places are likely to be affected by the policy and to determine which vulnerable peoples may be affected by or contribute to these environmental changes. Meanwhile, the purpose of mapping vulnerabilities is to develop a map of environmental and socioeconomic data to support the identification of vulnerable contexts to overlay probable policy impacts to further the analysis of environmental outcomes. The purpose of assessing the impacts is to determine how the policy will affect the environment in the vulnerable places and to describe the role vulnerable peoples are likely to play and also to estimate the significance of these impacts. Finally, the purpose of developing the recommendations is to understanding the geography of the impacts, the pathways of change and the environmental significance of the policy change provides the information needed to develop recommendations.

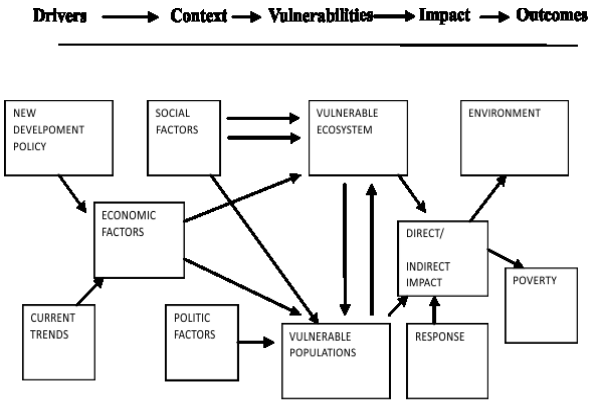

Figure 2: Impacts of Macroeconomic Policies Using a Vulnerable Based Approach.

A Vulnerability Based Approach.

The assessment may recommend a variety of measures, such as support for best practices, technology choices, fiscal measures, regulatory changes, infrastructure planning, payment for environmental services, resolution of tenure issues, development of community-based management options and support for protected areas. If the overall impacts of proposed policies are expected to be positive, recommendations will focus on strengthening or ensuring that positive impact. If negative impacts are likely, the assessment will identify alternative development policies or supplemental policies or programmes to ensure that poverty and sustainability objectives are met.

Vulnerable Populations.

Vulnerable populations refer to rural poor, indigenous communities and other groups that are heavily dependent on natural resources and unable to respond dynamically to economic change. These populations frequently depend on vulnerable ecosystems for their livelihoods. Any socioeconomic change that improves the ability of these people to manage resources sustainably will have environmental benefits; any socioeconomic change that reduces their ability to manage resources sustainably will strengthen the links between impoverishment and environmental degradation.

At the local level, poverty is usually a good indicator of human population vulnerability because it reflects exposure to socioeconomic and environmental stresses, high sensitivity to change and limited resiliency. Poverty in fact is particularly very useful as a proxy for vulnerability when poverty is measured in terms of livelihoods or well-being rather than strictly in terms of income. Poverty is a pronounced deprivation in well-being [7] resulting from a deprivation of a multifaceted set of material goods, assets, conditions and opportunities [8]. Furthermore, vulnerability of human populations is generally the results of a combination of negative factors that are closely related to economic poverty which include the overlapping factors such as poverty, limited control over resources, limited opportunities, food and livelihood insecurity and exposure to natural disasters.

4. A Case Study Description of an Existing Human Environment Introduction

This section describes the existing human environment at the proposed project site as well as the surrounding area. The description is based on primary data collected by the SIA consultant during the study period and supplemented by data from various studies carried out in the vicinity of the proposed site as well as published sources.

Population Distribution

The proposed project is located in Rimba Mas, Mukim Titi Tinggi, Perlis. The state of Perlis consists of 21 districts namely Titi Tinggi, Chuping, Beseri, Kurung Batang, Paya, Padang Sidin, Abi, Padang Pauh, Ngolang, Oran, Kechor, Sena, Sungai Adam, Jejawi, Wang Bintong, Kangar, Jejawi, Arau, Kurung Anai, Kuala Perlis, Seriab, Utan Aji, Kayang,and Sanglang.

Perlis has a population of 210,000 (Census 2000). The ethnic composition for the year 2000 are Malays with 174,805 (79.74%), Chinese with 21,058 (9.6%), Indian with 2,658 (1.21%) and Others with 20,690 (9.45%).

The objectives in population distribution are to collate and review all available data on population in the study area, with regards to size, age, sex, and ethnic group distribution of the local population; and to describe the population settlement patterns along the study area to include geographical distribution of existing living quarters and quality of houses.

The population distribution covers the Areas within 5 km radius surrounding the proposed area in Rimba Mas.

Socio-Economy

This section focuses on the relationship between economic activities and social life. In many cases, however, socioeconomics focus on the social impact of some sort of economic changes due to the development surrounding. Such social effects can be wide-ranging in size, anywhere from local effects on small community to changes to an entire society. The objectives of the socioeconomic are among others, to carry out a preliminary assessment of the existing

socioeconomic environment conditions that likely to be effected by the proposed project; and to gather local public opinions and views on the implementation of the proposed project.

Socioeconomic Survey.

In order to gauge the awareness and perceptions of the population within 5km radius and along the proposed pipeline, a socioeconomic survey was carried out. The study performed among others, the profiles of the impacted community in one way or another by the proposed project, assessed the probable impact of the proposed project on the socioeconomic, health and safety, aesthetic and cultural life of the community, provided recommendations for minimizing any adverse socioeconomic impacts of the development with particular attention to the surrounding areas, assessed impact that the proposed project on the scenic quality of the surrounding areas and assessed the suitability and possible impacts of the project on the workers during construction and on present health condition of the communities as a whole.
The study also addressed issues related to demography, socioeconomic resources and activities, public perception of the proposed project and infrastructure of the study areas.

Study Areas and Methodology

The survey was conducted in two different areas namely in Felda Rimba Mas and Kg Kubang Tiga which are located within 5 km radius from the proposed project.

Socioeconomic Background

Table 1.1 shows the 100 selected samples from villages or settlements which include Kg Kubang Tiga with 20 samples (20.0 percent) and Felda Rimba Mas with 80 samples (80.0 percent). In term of ethnic composition, 80.0 percents are Malays and 20.0 percents are Siamese.

Table 1.1: Distribution of Impacted Villages Within 5 Km Radius

Villages	Frequency	Percentage
Kg Kubang Tiga	20	20.0
Felda Rimba Mas	80	80.0
Total	**100**	**100.0**

In terms of the status of the respondents, there were 83.0 percent male household heads, 15.0 percent were women household heads and 2.0 percent were the wife to the head of households. It was indicated that, 94.0 percent of the respondents were married and 6.0 percent were divorced. With regards to the age-category, most of the respondents were within 55-65 age group (64.0 percent). Table 1.2 shows the details.

Table 1.2: Age of Respondents

Age Groups	Frequency	Percentage
38-39	3	3.0
40-44	5	5.0
45-49	7	7.0
50-54	20	20.0
55-59	39	39.0
60-64	24	24.0
65+	2	2.0
Total	**100**	**100.0**

The results also showed that 25.0 percent of the respondents finished primary school, and more than half (52.0 percent) had completed lower secondary education (Table 1.3).

Table 1.3: Educational Status of Respondents

Education Levels	Frequency	Percentage
Primary School	25	25.0
Lower Secondary School	52	52.0
Upper Secondary School	23	23.0
Total	**100**	**100.0**

Employment

Field survey and site observation revealed that the residents surrounding the proposed project areas are involved in various activities. Among the respondents interviewed, a significant number of them were Felda settlers (80.0 percent), paddy farmers (10.0 percent), traders (1.0 percent), factory workers (2.0 percent) and no permanent jobs (7.0 percent) as shown in Table 1.4.

Table 1.4: Main Occupation of Respondents

Occupation	Frequency	Percentage
No Permanent Job	7	7.0
Paddy Farmers	10	10.0
Petty Traders	1	1.0
Factory workers	2	2.0
Felda Settlers	80	80.0
Total	**100**	**100.0**

Table 1.5 and Table 1.6 show the distribution of monthly income and expenditure respectively among the respondents. The survey reported that almost 74.0 percent of the respondents' incomes were within RM1500.00-<RM2000.00 per month, and almost 80.0 percent of the respondents' expenditure fell within RM500.00-RM1000.00 income groups.

Table 1. 5: Monthly Income of Households

Income Groups (RM)	Frequency	Percentage
	15	15.0
500-999	6	6.0
1000-1499	74	74.0
1500-1999	5	5.0
2000+	**100**	**100.0**
Total		

Table 1.6: Monthly Expenditure of Households

Income Groups (RM)	Frequency	Percentage
400-499	4	4.0
500-599	20	20.0
600-699	22	22.0
700-799	12	12.0
800-899	6	6.0
900-999	10	10.0
1000-1999	26	26.0
Total	**100**	**100.0**

Utility and Amenity Infrastructures

The survey results indicated that almost all of the respondents lived in their own houses. The types of houses were mostly single storey village houses (80.0 percent) with good conditions (33.0 percent), and 67.0 percent were in moderate conditions. Most of the houses were supplied by pipe-water (100.0 percent) and electricity (100.0 percent). Meanwhile, the supply of home telephone was recorded only 68.0 percent, and may be due to the usage of hand-phone which was recorded up to 100.0 percent. As for sanitation facilities, 90.0 percent of the houses were equipped with flush toilets.

Table 1.7 shows the respondents' perception on basic amenities in the survey areas. It was found out that most of the items listed were responded as satisfactory by the respondents except for post office, secondary schools, market facilities, waste management, fire extinguisher, police control and recreational facilities were responded as unsatisfactory.

Table 1.7: Perceptions on Basic Amenities

Basic Amenities	Satisfactory (%)	Unsatisfactory (%)
Primary	81.0	81.0
School	0.0	0.0
Secondary	90.0	90.0
School	42.0	42.0
Public Phone	69.0	69.0
Post Office	92.0	92.0
Clinic/Hospital	39.0	39.0
Road	0.0	0.0
Bus/Taxi	98.0	98.0
Services	96.0	96.0
Market	97.0	97.0
Grocery Store	0.0	0.0
Mosque	0.0	0.0
Community	0.0	0.0
Hall	0.0	0.0
Waste Management		
Fire Extinguisher		
Police Station		
Recreational Facilities		

Awareness and Perceptions

Table 1.8 shows the respondents' perception on impact of the proposed project on their surrounding. It was found out that they perceived the major positive impacts would be on their jobs, income, daily life, real estate, standard of living, business and other economic activities.

Table 1.8: Respondents' Perception toward the Proposed Project

Perception Levels	Frequency	Percentage
Effect their job		
Yes	99	99.0
No	1	1.0
	0	0.0
Uncertain		
Effect their income	99	99.0
	1	1.0
Yes	0	0.0
No		
	100	100.0
Uncertain	0	0.0
Effect their daily life	0	0.0
Yes	92	92.0
No	8	8.0
	0	0.0
Uncertain		
Effect their real estates	92	92.0
	8	8.0
Yes	0	0.0
No		
	100	100.0
Uncertain	0	0.0
Effect their standard of living	0	0.0
	100	100.0
Yes	0	0.0
No	0	0.0
Uncertain		
Effect their business opportunities		
Yes		
No		
Uncertain		
Effect other economic activities		
Yes		
No		
Uncertain		

Most of the respondents gave very negative response when asked about the effect of the proposed project on the road accidents, traffic jam, spreading disease and security and other surrounding amenities and facilities. Table 1.9 shows the details.

Table 1.9: Effect of Proposed Project on Surrounding Amenities and Facilities

	Frequency	Percentage
Road Accidents		
Yes	100	100.0
No	0	0.0
	0	0.0
Uncertain		
Noise	100	100.0
Yes	0	0.0
No	0	0.0
Uncertain	100	100
Traffic Jam	0	0.0
Yes	0	0.0
No		
	100	100.0
Uncertain	0	0.0
Air Pollution	0	0.0
Yes		
No	100	100.0
Uncertain	0	0.0
Water Pollution	0	0.0
Yes		
No	100	100.0
Uncertain	0	0.0
Dangerous	0	0.0
Toxic		
Yes	100	100.0
No	0	0.0
Uncertain	0	0.0
Bad Odour		
Yes	100	100.0
No	0	0.0
Uncertain	0	0.0
Spreading		
Disease	100	100.0
Yes	0	0.0
No	0	0.0
Uncertain		
Security		
Yes		
No		
Uncertain		

As an overall evaluation, 90.0 percent of the respondents did not agreed that the proposed project would be very beneficial and bring advantages to the surrounding areas (Table 1.10). Majority of the respondents who disagreed indicated that the project would create negative impacts to the surrounding population.

Table 1.10 : Overall Evaluation

Advantage/Disadvantage	Frequency	Percentage
Advantage	2	2.0
Disadvantage	90	90.0
Uncertain	8	8.0
Total	**100**	**100.0**

5. Potential Impacts and Mitigating Measures

Potential Impacts and Mitigating Measures
Impacts and Mitigating Measures: Pre-Construction Phase.
Socio-economy
Introduction
The potential adverse impacts and beneficial impacts will be predicted on activities including construction of access work, site survey and investigation.

Potential Impacts
There will be some degree of significant potential adverse impact on human settlement and low degree of significant potential adverse impact on amenities and facilities.

Mitigating Measures
Proper disposal and management schedule wastes according to methods approved by DOE should be applied to all project activities in order to minimise dust, noise and the tranquillity to the surrounding area.

Impacts and Mitigating Measures: Construction Phase.
Socio-economy
Introduction
During construction phase, there will be a quite high expectation impacts on construction on access road to human settlement, amenities and facilities, trade and commerce and job opportunities.

Potential Impacts
There will be a potential adverse impact during the construction of access road.

Mitigating Measures
Adhere to the relevant guidelines of effective signage to convey safety and warning messages.

Potential Impacts
The construction work is expected to have a very significant adverse impact on the livelihood of the rubber tapers nearby because they will be unable to do their work due to the increase activities in the area.

Mitigating Measures
The adverse impacts on the local rubber tapers should be compensated by any compensated scheme agreed upon by the parties involved, with the consultation of the Public Work Department and local authority.

Impacts and Mitigating Measures: Settlement Stage.

Socio-economy

There will be no impacts and mitigating measures in the settlements such as Felda Rimba Mas and Kg Kubang Tiga nearby.

Policy Implications

- Public involvement through public dialogue. The dialogue should be open to the public at large and should no be limited to only for directly impacted segment of population.
- Assessment must be linked to the other components of environment such as fauna, flora and physical environment to include soil, hydrology and water.
- The evaluation should be focused according to the phase of development. If the evaluation has found that the problems will be specific to the vulnerable area, solution should be developed that target this area.
- Identified the methodology and assumptions before the starting of any survey or field work. This is important in order to avoid any policy makers from jeopardizing national policy objectives for the sake of local conservation goals.
- For the sustainability of the vulnerable people, indicators to reflect important aspects of changes should be considered in order to capture the evolving relationship between the vulnerable peoples and the environmental resources.

6. Summary

Socioeconomic section focussed on the social impact of some sort of economic change due to the development surrounding. The objectives are among others, to carry out preliminary assessment of the existing socioeconomic environment condition that are likely to be effected by the proposed project and to gather local public opinions and views on the implementation of the proposed project. There were 100 samples of household heads, taken as respondents, residing within 5 km radius from the proposed project. It was found out that 64 percent were within the economically active population and majority finished lower secondary school secondary school (52 percent). In terms of employment, 80 percent worked as Felda rubber tapers and 10.0 percent worked as farmers. Majority of the respondents earned income in the category of RM1500 to RM2000 per month and the monthly expenditure were between RM500-RM1000 per month. With regards to the awareness, 82.5 percent were aware of the proposed project and for overall evaluation, 90.0 percent of the respondents disagreed that the proposed project even though the project would be very beneficial and bring advantages as well as creating more job opportunities to the surrounding areas, due to the negative impacts.

The impacts and mitigating measures during the pre-construction stage was quite minimal on socioeconomic environment. It would be some potential adverse degree of significant on the nearby temporary rubber tapers settlement. The project proponent should take the proper disposal and waste schedule management together with the mitigating measures for noise in order to maintain the tranquillity of the nearby rubber tapers. The impact on socioeconomic environment is quite significant during the construction phase especially on human settlement. The mitigation should adhere to the relevant guidelines of effective signage to convey safety and warning messages. The construction work is expected to have a very significant adverse impact on the livelihood of the local rubber tapers nearby due to increase activities. As a mitigating measure, the local rubber tapers should be compensated with the consultation of Public Work Department and the Local Authority.

7. Conclusion

The vulnerable peoples live in places where the natural environment has been very seriously degraded due to the development of the area and where no environmental resiliency remains. By anticipating the potential environmental impacts and related social impacts of the new proposed project of NODE 1 in Nusajaya, Iskandar Malaysia, both the impacted communities in Kg Kubang Tiga and Felda Rimba Mas should not be neglected totally due to their vulnerability overlapping factors such as poverty, limited control over resources, limited opportunities, food and livelihood insecurity and exposure to natural disasters.

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Halal Sensors : The "Naqly-Aqly" Approaches

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Abstract - Currently, Muslims have become increasingly concerned about the food they consumed. Right and proper product description is very crucial for Muslim consumers to make informed ingridients and to ensure fair trade, particularly in the ever growing halal food market. Ideally according to Islamic law (the "nagly" approach), all food products should be produced by "Halal Build In" approach, it means that the food from farm to the table should be halal. However in real situation, we need "Halal Tested In" approach (the "aqly" approach). This is due to the fact that globally, Muslim consumers are facing a number of issues concerning food products such as alcohol content, pork substitution, pork derivatives, use of prohibited ingredients, and non-halal methods of slaughter in food products. These issues are encouraged and suitable analytical techniques are needed for the halal analysis, particularly simple, disposable, low-cost and user friendly tools and devices such as halal sensors. The halal sensors that are appropriate and specific have been developed to deal with particular issues in our lab. The most suitable sensors for any particular sample is often determined by the nature of the sample itself. This paper sets out to identify what makes food halal, highlight the halal authenticity issues that occur in food products and provide an overview of the possible analytical methods in particular halal sensor for halal authentication of food products.

1. Introduction

Halal is an Arabic term which means permitted, allowed, authorised, approved, sanctioned, lawful, legal, legitimate or licit. Guidelines for halal are given by Allah in the Holly Quran; "Forbidden to you (for food) are: Al-Maytatah (the dead animals — cattle-beast not slaughtered), blood, the flesh of swine...." (Surah Al Maidah, verse 3). Thus, in the Muslims' point of view, decision to choose one food over the other depends on its halal status. Muslims follow strict dietary laws according to the holy Quran. Historically, meat for Muslim consumers was not associated with adulteration and this could be attributed to the fact that it was sold fresh at easily recognisable products. However, now the food chain has become so long and modern lifestyles have changed greatly. This has resulted in the need to preserve and process food into various food products [1]. With technological advances in the food processing industry, adulteration and fraud have become common due to economic benefits.

As consequences halal verification and authenticity is an issue of major concern in Muslim consumer products and among the issues is related to alcohol content for halal verification and pork based adulteration such as porcine, lard and others for halal authentication. In the Islamic point of view, alcohol and pork or lard are serious matters and totally prohibited in consumer products. As consumer products are part of our daily life, Islamic Laws carry a special significance as mentioned in the Holy Quran. In Islam, food containing pig components are *haram* (prohibited or unlawful) for Muslim consumption [2]. From a nutritional point of view, amongst all animals, pig is the cradle of harmful germs and in addition, pork serves as a carrier of diseases to mankind. It is for those reasons that made it unhealthy for human consumption [3].

On the other hand, alcohol/ethanol due to its low toxicity effects, can be consumed by human. Ethanol is the main constituent found in alcoholic beverages and other products that undergo fermentation. Besides that, ethanol also can be found in non-food products such as mouthwash, cosmetic products and medicine. Alcoholic drinks or beverages are totally prohibited in Islam, and even a small amount of the drink added into foods or drinks will render the products *haram* [4]. However, trace amounts of ethanol (naturally present or ethanol used in food processing) is allowed if the amount is insufficient to cause intoxication (less than 1%)[5].

Hence, developing analytical methods for halal verification and authenticity are very important especially for the Muslim consumers to protect them from adulteration and fraud and also to ensure products safety and quality. Conventional methods, such as chromatography (HPLC & GC-MS) have been developed [6] and spectroscopic methods (FTIR) have been developed [3] followed by a rapid, short column chromatography technique. However, they need that the samples have to be sent to laboratory to analyze the presence of alcohol and pork content. Usually, the process takes weeks and is very tedious. In addition,

such methods are time consuming, constitute a source of error and discrepancies between laboratories, as well as need skilled personnel for operation of those expensive instruments [7]. Therefore, the application of alternative methods for Halal determination is needed, which make the analysis can be simplified. One can easily detect the presence of alcohol and pork components within minutes and this will be very useful to the Muslim enforcement in determining the safety consumption of the products.

In this review, we focused on the current technology of halal sensors as a tool/device for halal verification and authentication that can be used for consumer products. In the first part, the review has been focused on the alcohol (ethanol) sensors based on chemical sensor and enzyme based biosensor for halal verification. The second part, the work review has been focused on the development of pork sensor (e.g, based on antibody and DNA). These pork sensors could be applied for detection of pork or pork related products (e.g. porcine, lards, gelatine etc) as halal authentication. In the final part, the review has been focused on the possibility to use nanotechnology in development of halal sensor in this emerging need that has great potential in the development of new sensing devices for assessing halal products in the near future. Furthermore, these will be very useful to the Muslim enforcement in determining the safety consumption of those consumer products.

2. Alcohol Sensors

2.1. Optical Sensors

Alcohol as organic solvents are typically completely transparent in the visible region, but exhibit strong absorptions in the NIR (near infra red) region (measured in a 1-cm thick cuvette) [8]. While their NIR spectra are quite similar, the composition of a mixture of such solvents can be identified with high accuracy by means of chemometric analysis. Using this NIR method, we could develop optical sensor for alcohol detection in couple with chemometric analysis such as partial least square, principle component analysis/PCA etc.

The optical set-up of NIRS for alcohol detection could be seen in Fig. 1, where the detection could be performed using non-destructive method. Thus, this technique is suitable to be used for alcohol detection in various samples e.g. food and other consumer products.

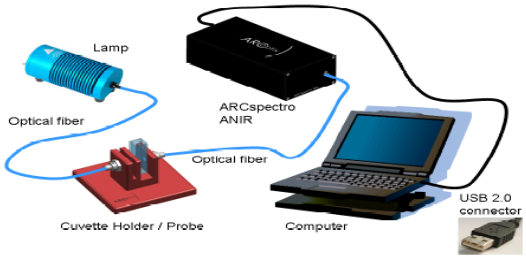


Figure 1. NIRS set-up for alcohol detection and their spectra toward alcohol (Arcoptix, 2011).

Organic solvents, such as alcohol are typically completely transparent in the visible region, but exhibit strong absorptions in the NIR (near infra red) region (measured in a 1-cm thick cuvette) as given in Figure 2 (Arcoptix, 2011). While their NIR spectra are quite similar, the composition of a mixture of such solvents can be identified with high accuracy by means of chemometric analysis such partial least-squares (PLS) calibration and linear discriminant analysis (LDA). Currently, this method is developed in our lab for pharmaceutical products.

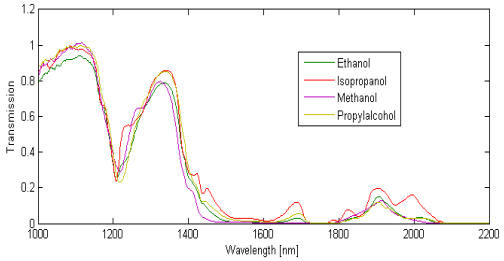


Figure 3. NIR spectra of alcohol (ethanol, isopropanol, methanol and propylalcohol).

2.2. Chemical sensor

Detection of alcohol (ethanol) could be detected using dichromate reagent (potassium dichromate) based colorimetric method and their reaction time (kinetics). Here, ethanol will oxidised to became aldehyde and carboxilate acid as given in the reaction below.

$$K_2Cr_2O_7 + 3\ CH_3CH_2OH + 16H^+ \longrightarrow 3CH_3COH + 2\ Cr_20_7^{2-} + 22H^+ \ldots..(1)$$

Dichromate ($Cr_2O_7^{2-}$, Cr (VI)) has orange color and in reduction form by ethanol become green color (Cr^3, Cr(III)). Based on their reaction kinetics, the concentration of etanol in the sample will affect to ther rate of the reaction in term of their color cahnge from orange to green. As a results, in high concentration of etanol, the rate of color change will be faster compared to less ethanol concentration [9]. Based on this principle, we could develope method for ethanol concentration based on the rate of color change (reaction rate) or steady state, in simple method for

ethanol detection. Based on this method, chemical sensor based on dry reagent of dichromate has been developed in our lab for ethanol detection in various samples as given Fig. 4 below.

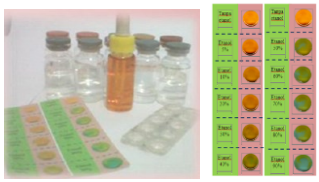


Figure 4. Alcohol test kit based on dicromate reagent (left) with color comparator (right).

2.3. Enzyme based biosensor

Ethanol can be made by the fermentation of sugars, and it is also of all alcoholic beverages. Fermentation is usually carried out by adding yeast to a mixture of sugars and water. Yeasts contain enzymes that promote a long series of reactions that ultimately convert a simple sugar to ethanol and carbon dioxide:

$$C_6H_{12}O_6 \xrightarrow[95\%\text{ yield}]{\text{yeast}} 2\,C_2H_5OH + 2\,CO_2 \quad (2)$$

Fermentation alone does not produce beverages with ethanol content greater than 12-15% because the enzymes of the yeasts are deactivated at higher concentrations. To produce beverages of higher alcohol content the aqueous solution must be distilled. The flavors of the various distilled liquors result from other organic compounds that distill with the alcohol and water. Ethanol is a hypnotic, depresses activity in the upper brain even though it gives the illusion of being a stimulant. Ethanol is also toxic, but it is much less toxic than methanol. Abuse of ethanol is a major drug problem in most countries [10].

Alcohol oxidase catalyzes biological oxidation of short chain; primary, aliphatic alcohols to the respective aldehydes while molecular oxygen is reduced to hydrogen peroxide. Enzymatic assay of alcohol oxidase is described below:

$$\text{Methanol} + O_2 \xrightarrow[\text{AlcOx}]{} \text{Methanal} + H_2O_2 \quad (3)$$

$$H_2O_2 + \text{ABTS} \xrightarrow[\text{POD}]{} 2H_2O + \text{oxidized ABTS} \quad (4)$$

Where: AlcOx: Alcohol oxidase, POD: Peroxidase and ABTS:2,2'-Azino-bis(3-Ethylbenzthiazoline-6-sulfonic acid). The absorbance is measured at 405 nm and the intensity is correlated with the amount of product [11].

The enzyme has the highest affinity for methanol with the affinity decreasing with increasing chain length of the alkyl (R) group. Alcohol oxidase plays a major role in the metabolism of methanol resulting in the formation of formaldehyde and has been detected in several genera of yeasts, such as *Candida*, *Pichia*, and *Hansenula* that utilize methanol as a sole carbon and energy source. Alcohol oxidase can be used in alcohol detection in blood or food analysis. AlcOx based biosensors are straightforward since they function (using molecular oxygen as the cofactor) without any addition of cofactor such as nicotinamide adenine dinucleotide or flavin adenine dinucleotide (NADH or FADH). As shown in Figure 4, the enzyme requires molecular oxygen to oxidize ethanol to acetaldehyde, and product formation is amperometrically detected.

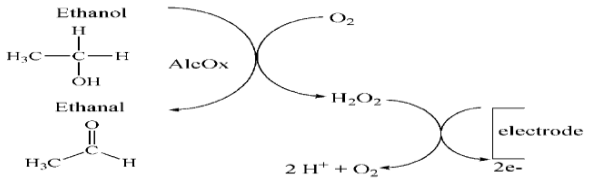


Figure 5. Mechanism for non-mediated amperometric ethanol sensing based on monitoring hydrogen peroxide production.

Alcohol oxidase catalyzes biological oxidation of short chain; primary, aliphatic alcohols like ethatanol to the respective aldehydes while molecular oxygen is reduced to hydrogen peroxide as described below:

$$\text{Ethanol} + O_2 \xrightarrow[\text{AlcOx}]{} \text{Ethanal} + H_2O_2 \quad (5)$$

$$H_2O_2 + \text{ABTS} \xrightarrow[\text{POD}]{} 2H_2O + \text{oxidized ABTS} \quad (6)$$

Where: AlcOx: Alcohol oxidase, POD: Peroxidase and ABTS:2,2'-Azino-bis(3-Ethylbenzthiazoline-6-sulfonic acid). The absorbance is measured at 405 nm and the intensity is correlated with the amount of product [11].

The enzyme has the high affinity for ethanol with the affinity decreasing with increasing chain length of the alkyl (R) group. Alcohol oxidase plays a major role in the metabolism of ethanol resulting in the formation of ecetaldehyde. Alcohol oxidase can be used in alcohol detection in various samples (food and pharmaceutical analysis). AlcOx based biosensors are straightforward since they function (using molecular oxygen as the cofactor) without any addition of cofactor such as nicotinamide adenine dinucleotide or flavin adenine dinucleotide (NADH or FADH). As shown in Eqn. 6, the enzyme requires molecular oxygen to oxidize ethanol to acetaldehyde, and product formation of hydrogen peroxide is amperometrically detected or optically detected via conducting polymers (e.g. polyaniline) as in our work [12] as given in Fig.6. Based on this method various enzyme based could be developed using electrochemical or optical techniques. The review on enzyme based alcohol biosensor has been published resently by Kuswandi & Ahmad [13].

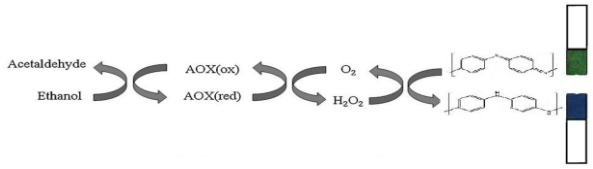


Figure 6. Proposed reaction mechanism at AOX/PANI film based biosensor towards ethanol [12].

3. Pork Sensors

3.1. Optical Sensors

Spectroscopic methods are attractive options due to the speed of analysis and minimal sample preparation. Near Infrared Reflectance Spectroscopy (NIRS) was originally developed to provide a rapid measurement of the composition of grains and oilseeds[14]. NIRS has emerged in the last 30 years as a rapid method for testing the quality of intact samples from the light they reflect [14,15] and it is likely to be the best means of achieving this quality on troll effectively and conveniently. Most of the established methods have involved the development of NIRS calibrations for the quantitative prediction of composition in meat [16,17]. This was a rational strategy to pursue during the initial stages of its application, given the type of equipment available, the state of development of the emerging discipline of chemometrics and the overwhelming commercial interest in solving such problems[18] .

The typical spectra of beef, sheep, pork and chicken muscle samples in the VIS and NIR regions are shown in Figure 7. Visual differences were observed between sample species in the visible region (respiratory pigments) and in the near infrared region (fat content) [19]

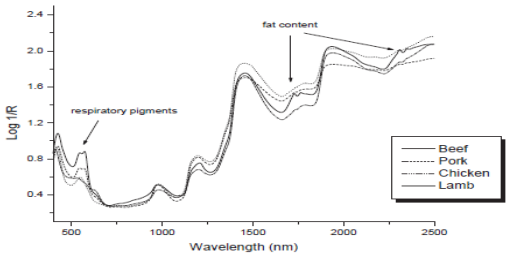
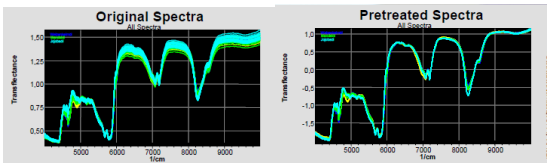


Figure 7. Visible and near infrared mean spectrum of pork, beef, lamb and chicken samples.



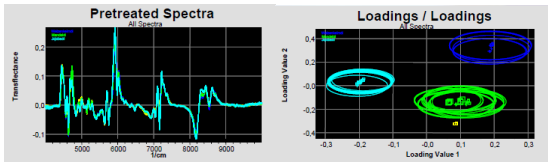


Figure 8. General procedure for their spectra discrimination with chemometric [8].

The typical optical sensor based on NIR for identification spectra of certain samples (e.g. beef, sheep, pork and chicken muscle samples) are shown in Fig. 7 including the application of chemometric in Fig. 8. Visual differences were observed between sample species in the visible region (respiratory pigments) and in the near infrared region (fat content).

3.2. Immunosensors

Antibodies that retain their antigenicity could also be developed as immunosensor to determine pork components/derivatives in food or pharmaceutical product. The utilizing a biotin-avidin for antibodies immobilization will enhance the detection process. With increased concentrations of pork-specific protein in the extract, more of the protein will bind to antibody attached to the sensor surface as given in Fig. 9.

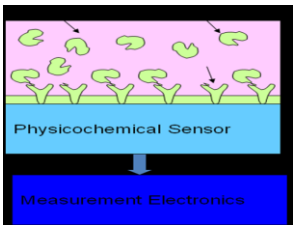
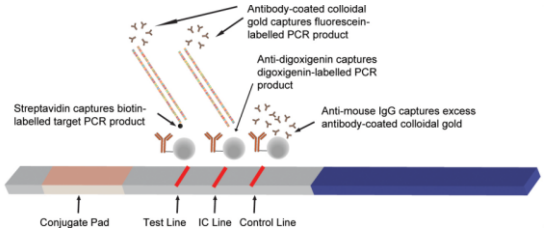


Figure 9. The structure of immunosensor based on analyte-specific protein

Here, analyte (e.g. porcine or lard) will be probed by a specific affinity reaction that binds it to a biomolecule on a sensor, where attachment changes interface properties. Commonly used sensors: Gravimetric (i.e. quartz crytsal microbalance and surface acustic wave), electrochemical, and optical method.

Main method for species identification on 1990s period was based on the properties of the proteins [20]. In these techniques, proteins are extracted from beef jerky or shredded meat with solvents and subjected to electrophoresis or immunological analysis. Typical immunological methods rely on diffusing the extracted proteins of interest against antibodies to proteins from a range of different animal or plant species. Adulteration test on meat product were so difficult, especially heat processed product like beef jerky and shredded meat, heat process resulting denaturized proteins [21]. Therefore, antibodies to heat-stable soluble proteins,

which retain their antigenicity after high temperature process, must be prepared [22]. The immunosensor in dip stick format is given in Fig, 10.



Fig, 10. Immunosensor for dipstick fromat.

3.3 DNA Biosensors

Initial studies using DNA to detect meat species used relatively simple methods, whereby labelled DNA probes were hybridized to samples of genomic DNA covalently attached to nylon membranes in a slot- or dot-blot format [23, 24, 25,26]. In this most basic regime, it was shown that under conditions of appropriate stringency and for certain species, probes comprising labelled total genomic DNA from a given species would hybridize to DNA from the same species with little cross-reactivity. The preparation of such probes did not require any prior knowledge as to the precise nature of the DNA sequences under study. Species specific binding of the probes to targets was believed to result from the hybridization of complementary repetitive sequences [25]. These are tandemly-arranged and found throughout the genome as `satellite' sequences [Fig. 11].

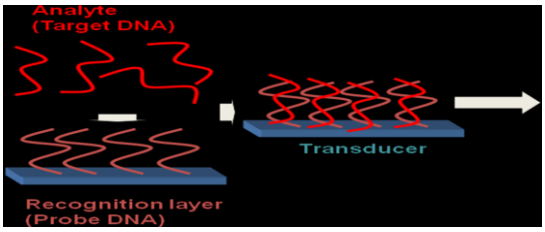


Figure 11. Principle of DNA biosensor based on hybridisation reaction.

Using such methods, samples of chicken and pork, DNA extracted from cooked meats and commercial products [23, 25, 26] and pork in pork/beef mixtures [24] were unequivocally identified. Probes for goat, sheep and beef were demonstrated albeit, with some degree of cross-reactivity [23, 26]. Such results were liable to be a reaction of the varying degrees of homology in the sequences of the satellite DNA in the deferent species.

Fig. 11 shows general structure of DNA biosensor based on hybridization scheme. Here, DNA hybridization biosensor will consist of immobilization of ss-DNA probe onto the transducer surface, and then transducing (association of an appropriate hybridization indicator). The detection method could be electrochemical (e.g. ccurrent signal of a redox indicator), optical (emission signal of fluorescent or chemiluminescent labels or surface optical properties/SPR or nanoparticle based colorimetric detection) and mass detection (QCM, frequency signal of oscillating crystal with DNA probe).

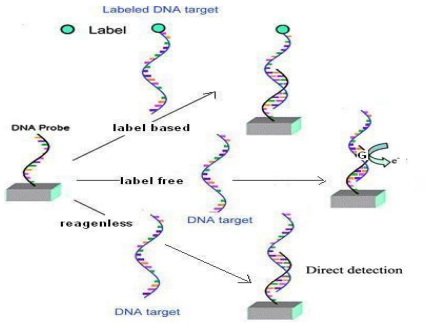


Figure 12. The transducing principles of DNA hybridization biosensor (label-based, label-free, and reagentless schemes).

All these methods will be explored in order to find best DNA hybridization biosensor for detection of swine components and its derivatives in various food and consumer products. With the development of DNA hybridization biosensors in the food, pharmaceutical and health products, the analysis can be simplified. They can easily detect the presence of pork components within minutes. Thus, these will be very useful to the Muslim enforcement in determining the safety consumption of those products as well as for Halal verification.

4. Summary

Scientifically, the halal issues need to be supported with adequate tools so that the consumer products could be verified or authenticated as halal or haram products. Thus, halal verification and authenticity of the products are an issue of major concern in the Muslim community. Therefore, the development of tools or small device that can be easily used by common people in the field for halal assessment is extremely important. One of alternative that can be used for this purpose is sensor technology, in particular chemical sensors and biosensors. Current sensor technologies offer useful devices for development of halal sensor for foods and consumer products. Chemical sensors based on host-guest chemistry, could be designed as halal verification for detection of alcohol content as target analyte. While, biosensor based on bio-receptor (e.g. antibody and DNA) could be designed as halal authentication for detection pork or pork related products as target analyte. The emerging of nanotechnology will also add to advancement of this sensor technology that can be used in future development and application.

5. Acknowledgments

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Acquiring Analytical Skills in Approaching Bioethical Issues: The IIUM Experience

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Abstract – Analytical skills in bioethics are important learning objectives of medical education at the Kulliyah of Medicine, International Islamic University Malaysia since its inception in 1997[1]. Small group discussions, carried out during the two-week posting in Anesthesiology and Critical Care is designed to enhance students' analytical skills in bioethical issues, in particular the application of the contemporary and Islamic ethical theory of *Maqasid* and *Qawaid al-shariah* to solve ethical dilemmas of common situations that are encountered in clinical practice. Students are tasked to prepare the discussion script of given Case Studies as stipulated in the "Guidebook on Discussion of Medical Ethic" before the small group discussion session. Ethical issues that are discussed include informed consent, DNR, withholding and withdrawal of treatment, euthanasia, brain death, organ donation and end of life care. This method of learning received good feedback from students.

1. Introduction

Ethics generally refers to a science concerning the question of right and wrong in the human conduct, a normative science dealing with, *"how things ought to be"*. Ethics connotes deliberation and explicit arguments to justify particular actions. It also refers to a branch of philosophy that deals with the "principles governing ideal human character" or a professional code of conduct. To philosophers, ethics focuses on the reasons why an action is considered right or wrong. It asks people to justify their positions and beliefs by rational arguments that persuade others. Medical ethics is a field of applied ethics whereby moral values and judgments are applied in decision-making process. It is defined as the study and application of moral values, rights and duties in the field of medical treatment and research. It is an analytical activity in which concepts, assumption, beliefs, attitudes, emotions, reasons and arguments underlining medico-moral decision-making are examined critically. It is one of the main attributes of professionalism that must be developed. Thus, an appropriate learning activity is required to help students develop the skill of analyzing ethical issues that are present in difficult cases, by applying the appropriate ethical principles, both contemporary and Islamic to arrive to a satisfactory solution.

2. Contemporary Medical Ethic

The modern-day medical ethics was attributed to Thomas Percival, an English Physician, who wrote *Code of medical ethics* in 1803 which emphasized care and attention to aspire patients with gratitude, respect and confidence. He opined that doctors are permitted to withhold the truth if it would be deeply injurious to the patient. It is the very nature of medical decisions that value choices must be made constantly, which can be very challenging. Deciding on trivial situations such as deciding for an emergency appendicectomy is generally easy, but not on situations of life and death such as deciding not to perform cardiopulmonary resuscitation on a patient who is literally 'dead' on arrival at the A&E or withdrawing a life support machine from a ventilated patient who shows evidence of brain death.

The contemporary scientists and medical professionals generally adopt the four basic ethical principles that was popularized by Beauchamp and Childress; namely, autonomy, beneficence, non-maleficence and justice. The principle of autonomy is the power of the patient to decide on medical procedures. The principle of non-maleficence is avoiding causation of harm while beneficence is the provision of benefits and balancing them against risks and costs. The principle of justice is distribution of benefits, costs, and risks fairly. Unfortunately it is not wholly effective because it is neither law enforced by the authority of the state nor morality enforced by conscience.

3. Islamic Medical Ethic

Islamic philosophy has contributed a lot in the development of science and medical ethic during the Golden Era of Muslim civilization. The guiding principle in Islamic medical ethic is mentioned in the

Quran and also in the Torah is, "If anyone has saved a life, it would be as if he has saved the life of the whole mankind". Islam differs from others in that

ethics is part of its Law *(Shari'ah)*. The Islamic term corresponding to this concept, though different in scope and nature, is *ilm al-alkhlaq*, a branch of knowledge which deals with the way to maintain virtues as their optimum level, i.e. to avoid wrongdoing and to do what is right and desirable. From the Islamic perspective, the scope of *akhlaq* covers the relationships of Man-God, Man-Man and Man-Environment that a believer should adhere at all time. *Akhlaq* in a broad sense subsumes all actions that are characterized as *a'mal salih* in the terminology of the Quran.

Strictly speaking, there is no need for a separate "medical ethic" for Muslim physicians as Islam considers medical ethic the same ethic as in other areas of life, which is derived from the syari'ah or the Law. In order to speak in the same language with the rest of medical fraternity, the so called medical ethic in Islam would be best described as a mere reinstatement of general ethical principles using medical terminology and with medical applications. It is important for students to understand both the contemporary and Islamic ethical theories and principles, in particular the *Maqasid* and *Qawaid al-sharia'ah* and apply them in resolving common ethical dilemmas in clinical practice.

The basis of Islamic ethical theory is found in the five purposes of the Law, *maqasid al shari'at*, which are the preservation of faith, (*diin*), life, progeny, intellect, and wealth. According to this theory, medical practice and/or procedures must not contravene any one of the above purposes if it is to be considered ethical. On the other hand, the basic ethical principles of Islam relevant to medical practice are derived from the 5 principles of the Law which are: intention, (*qasd*); certainty, (*yaqeen*); harm, (*dharar*); hardship, (*mashaqqat*); and custom, (*'aadat*). Thus, ethics cannot be divorced from the Law, and the human mind, guided by the Law, shariah is capable of working out rationally what is right and what is wrong for most problems in life. The reasoning used in formulating the ethical principles should always be guided by revelation, (*wahy*), and the saying of the Prophet pbuh to reach to the correct conclusions. Any medical reasoning and decision must fulfill one of the above purposes if it is to be considered ethical and decisions would be deemed unethical if it violates any of the five purposes of Law. Another outstanding feature of Islamic teaching that is relevant to medical ethics is avoidance of doubtful things. The prophet taught us to leave what causes us to doubt from what does not cause such doubt, (*da' ma yuribuuka ila ma la yuriibuka*). Generally, Islamic Medical Ethics agrees to the four basic principles of biomedical ethics; respect for the autonomy, beneficence, nonmaleficence, and distributive justice but it has to be interpreted within the framework of the Law, syariah. In Islam, principle of autonomy does not allow one to have freedom to choose what he/she wants. For example, taking one's own life as in euthanasia is unlawful, haram. Promoting benefit and preventing harm is encouraged in Islam but the circumstances of what is beneficial should be analyzed carefully so as not to contradict the maqasid and qawaid-al shariah. For example, issuing a DNAR order to a terminally ill patient is considered lawful according to the Maqasid al-shariah, when one is convinced that nothing could be done to prolong the patient's life and thus allowing death to occur as predestined by Allah, ajal. In this circumstance, the preservation of wealth (in terms of cost of medical care and treatment) takes priority over preservation of life and intellect. Insisting to prolong life in such situation is non-justice and thus unethical according to the Maqasid al-shariah. The Islamic ethical system provides consistent, comprehensive and practical guidelines to healthcare professionals in resolving bioethical dilemmas that are encountered in medical practice.

4. Acquiring Competency in Medical Ethic

Knowledge in medical ethic is acquired through didactic lectures during the pre-clinical year and as student seminars, small group discussion, bedside teaching and case reports during the clinical year. As students went through the medical training, they need to acquire the skill of applying the ethical theories and principles in resolving ethical dilemmas. One of the methods of acquiring this skill is through small group discussions of those common ethical dilemmas such as being carried out in the fourth year, during the two-week rotation in anesthesiology and critical care. Normally, eight to nine students rotate every fortnight to complete the posting. For the learning activity on medical ethic, students receive the *"Guidebook to discussion of medical ethic"* to guide the learning process that aims to assist them to understand the ethical theories and principles (contemporary and Islamic medical ethic) and their applications in difficult clinical case scenarios (which are real cases, not fictitious) in anesthesiology and critical care.

The Case Scenarios discussed are as follows:

1. Organ procurement and donation
2. Discontinue CPR
3. Withdrawal of life support
4. Consent of treatment by doctors
5. Disclosure of a misadventure – How to do it?
6. Respect the consent

Each student prepares a topic from the above list and delivers it during the discussion session. The remaining students are tasked to discuss on topics such as euthanasia, end-of-life care and pain management in the critically ill patients. Two clinical scenarios are provided as examples for students to understand how one could apply the ethical theory and principles in making decision of difficult medical situations.

4.1 Application of ethical theories and principles

The following two examples demonstrate the thinking framework that guide one in the decision-making process. Following these examples, each student prepares his/her answer script of the assigned topic, which will be presented during the small-group interactive discussion.

4.1.1 Example 1

Mr X, an otherwise healthy 24 year-old man, is diagnosed to have an acute appendicitis and is scheduled for an emergency surgery.

Principle of Justice

Imagine that you are the doctor managing this man and you find that he is very anxious as well as worried about his condition. His main concern is to get well as soon as possible and return to his normal life. As a good and safe doctor, informing him about the need to undergo surgery is justice done according to the current best medical practice.

Principle of beneficence and non-maleficence

Explaining the benefit or advantageous of the proposed surgery is the application of the principle of beneficence. The surgery is proposed with the intent of doing good thing for him. It is also required to tell him that, although there is a possible harm from surgery such as infection and complications related to anesthetic care, he is assured that it is going to be performed by experts (i.e.the surgeon and the anesthesiologist) and this constitutes the application of the principle of non-maleficence.

Principle of autonomy

This principle requires that the patient is given the autonomy of thought, intention and action when making his decision, after explanation by the doctor. The patient himself must decide whether to accept or otherwise, to the suggestion by the doctor. If he agrees then he consented for the surgery and indicates his agreement by signing in the consent form. Independent decision by the patient in terms of thought, intention and action is the application of the principle of autonomy.

Application of the maqasid al-shariah

The emergency surgery is proposed as it is the best chance for him to return to his normal pre-morbid life and continue his obligation as a responsible Muslim. Agreeing for surgery is necessary for the preservation of life and intellect. Getting back to his job to earn his income constitutes to the preservation of the five items of the maqasid al-shariah.

Application of the qawaid al-shariah

The first principle of the qawaid al-shariah applies when the doctor expresses his intent that surgery is needed and he is certain or yaqeen that the harm would be removed, and this is the application of the second principle of the qawaid al-shariah.

4.1.2 Example 2

Mr H, a 67-year old Muslim man, presumably had a heart attack while watching television with his wife at their house. He then became unconscious and stopped breathing. No CPR was performed on him. His wife managed to get him to the nearby hospital about half an hour later. Let say, you are the doctor on-duty who received him and noted that he was cyanosed, unconscious with an absent pulse and no spontaneous breathing. The Medical Assistant in your team connected the ECG to him and noted an agonal rhythm. Would you do a CPR on him?

Principle of justice

It is important to remember that ethical decision is medical and all medical procedures are considered permissible unless there is evidence to prove their prohibition. In such a situation, since no CPR was performed at home and there are evidences that death has already occurred by the time he reached the hospital, not performing a cardio-respiratory resuscitation is justifiable.

Principle of beneficence and non-maleficence

Instituting a CPR is not going to benefit him as he came only half an hour later. It is a known fact that irreversible neuronal damage is almost certain if no CPR is performed ten minutes after cardiac arrest has occurred. Insisting to perform a CPR may harm him further and this contradict the principle of non-maleficence. Furthermore, providing CPR gives false hope to the family member that he might survive, when in actual fact he is already dead the time he reached the hospital A&E.

Principle of autonomy

The decision not to perform a CPR in this case is justice done to this patient, which must be explained to the family members. Every ethical decision is a medical endeavour and no other parties shall interfere with the doctor's decision. A doctor is duty-bound to break the bad news and ensures that the family member understand the rationale of the decision that was taken. The family members should have the autonomy to agree or disagree with the explanation given by the doctor and finally indicate their decision.

Application of the maqasid al-shariah

In this case scenario, as the condition is considered medically futile and that CPR is unjustifiable, what is left for consideration of the maqasid al-shariah is the preservation of wealth. Wastage of human resources to revive a medically futile person is unjustified and unethical.

Application of the qawaid al-shariah
The principle of certainty, yaqeen allows one not to perform the procedure (i.e. withholding CPR) if one is certain that such action is unnecessary and is supported by various studies (evidence-based medicine)

The detail of this learning activity is discussed in the 'Student Guidebook to Discussion on Medical Ethic'. All these learning experiences provide opportunities to develop analytical skills in medical ethic that would enable them to practice holistic patient care as good doctors. Evaluation forms were distributed at the end of discussions to determine the general perception and effectiveness of the learning approach.

5. Result

Understanding and application of the contemporary ethical and Islamic ethical principles through preparation of task specific Case Study followed by two-way interaction with a teacher in a small group discussion were greatly appreciated by students. However, some discussion sessions tend to be a 'mini lecture' with minimal interaction from the students. This was probably due to inadequate knowledge and clinical experience of the students and lack of motivation as competency in bioethics is not taken into account in the overall assessment of their personal and professional development. Perhaps, with the introduction of portfolio assessment as part of the continuous assessment of the final professional examination overall mark would motivate students to take this learning experience more seriously. This approach will be in place in the next academic session.

6. Conclusion

It is essential that medical students acquire the analytical skills in solving ethical dilemmas and be familiar with the four ethical principles of medical ethic and also the *maqasid* and *qawaid al-shariah* that are used to approach clinical ethical dilemmas. Small group discussion of ethical issues in anesthesiology and critical care, as well as in lectures, seminars, bedside teaching and case reports are among the methods used to enhance the skill and competency in medical ethic among medical students. Hopefully, these learning experiences would enhance their confidence and commitment to perform their duties as good doctors sought by patients in the future.

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The Community Health Posting: Experience of Universiti Sains Islam Malaysia (USIM)

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Abstract - The Community Health Posting teaching module has been incorporated into fourth year medical student in Universiti Sains Islam Malaysia (USIM). The objectives of integrating Islamic principles and values in the medical curriculum that is emphasized in Community Health Posting are appreciating and understanding the medical and *fiqh* aspects of health and disease, understanding the social issues in medical practice and research and maintaining Islamic professional etiquettes. This teaching module illustrates the relevance of humanities in understanding illness and medical care within the community. Teaching and learning activities enable the students to explore a wide range of influencing factors and how these affect each patient and his/her family within psychosocial and underlying community and ecological perspectives. This posting utilizes various teaching and learning techniques such as lectures, tutorials, seminars, group discussions, educational visits, practical sessions and bedside teachings. In general, the students have acquired Islamic knowledge through the integrating of Naqli and Aqli components in the Community Health Posting.

1. Introduction

The Community Health Posting is a nine-week posting in the fourth year of the Bachelor of Medicine and Surgery (MBBS) programme conducted by the Faculty of Medicine and Health Sciences (FPSK) of USIM. During this posting, the core knowledge of public health, which include basic concept of public health, epidemiology, medical statistics, demography, family health, environmental and occupational health, health promotion and nutrition will be reinforced through experimental learning. The uniqueness of Community Health posting for USIM MBBS programme is that this posting adopts the humanistic approach and Islamic principles and values in line with the mission and vision of the Faculty. Vision of the FPSK of USIM is upholding the various fields of medicine and health sciences is the prerequisites for meaningful contribution to the society and mankind. The mission of the Faculty is that the Faculty strives to become a leading institution that promotes and integrates Islamic values in acquisition of knowledge in the fields of medicine and health sciences and be at the forefront in solving contemporary issues concerning the society and Islam through utilization of current approaches and technologies.

Omar Hasan Kasule presented a paper entitled 'Concepts of Islamic Medical Education' at a conference organized by the Islamic Medical Association of Malaysia at Khota Bharu in Kelantan in June 1996 where he mentioned about six (6) conceptual issues in Islamic Medical Education. The conceptual issues were the purpose of medicine, integration of the curriculum, the service vocation in medicine, physician leadership role, medical research as *ijtihad*, and physician motivation [1].

2. The Community Health Posting in University Sains Islam Malaysia (USIM)

The Community Health Posting of FPSK USIM is a continuation of Community Health subjects that had being taught to the medical students in Year 3. The practical aspect of Community Health, community based research and intervention programme, district hospital attachment as well as visit to various health related institution had been incorporated into the 4th year of the medical training. During this posting, the students are sent to the district of Tampin in Negeri Sembilan for attachment. This posting is divided into two main attachments, one community-based study with its intervention programme and several educational visits. This posting utilizes various teaching and learning techniques such as lectures, tutorials, seminars, group discussions, educational visits, practical sessions and bedside teachings. At the end of the Community Heath Posting, students are expected to know functions of District Health Office and Public Health Organizations and its functions, analyze community health problems of a selected

community following a health survey, organize health intervention programme and health promotion activities based on the formulated community diagnosis and lastly the students are expected to master the skills of effective communication and taking into consideration Islamic values when dealing with patients and members of the community.

3. Integration of Naqli and Aqli Components in the Teaching Module

The first revealed verses of the Qur'an state: *Recite in the name of your Lord who created. Created man from a clinging substance. Recite, and your Lord is the most Generous. Who taught by the pen. Taught man that which he knew not* [2]. Thus, Islam indeed encourages its followers to seek knowledge, and it is the religious duty of the Muslims to seek and disseminate knowledge. Medical knowledge is an important knowledge and it is a collective duty of a Muslim physician to teach other Muslims to be good in the field.

The objectives of integrating *Naqli* (revealed knowledge) and *Aqli* (by reasoning) and Islamic values in the medical curriculum that is emphasized in Community Health Posting are appreciating and understanding the medical and *fiqh* aspects of health and disease, understanding the social issues in medical practice and research and maintaining Islamic professional etiquettes.

3.1 Exploration of Socio-cultural and Spiritual Aspects in Patients' Community-Based Case Study

Understanding of the medical condition and psychosocial aspects of the patients studied is emphasized in this module. Attention is given to several areas of the patient's present situation – attitude and coping strategies, family behaviour in relation to the sick individual and the living environment. Without sound clinical knowledge, the explaination of diseases to the patient is impossible. The students are expected to discuss with the patient regarding the disease from Islamic point of view, whereby the acceptance of the disease is very important. The diseases are tests created by Allah so that Muslims will long for heaven, contemplate the blessings, the gift of health granted to them by Allah and give thanks to Him and also realize how weak they are, which should make them surrender to Allah with all their hearts. Muslims patients need to understand that the occurrence of disease as qadar. It is Allah's pre-destination that a person falls sick. Treatment and prevention of disease not against qadar. Medical treatment is subsumed under the principle that qadar can reverse another qadar. Allah had stated in the Quran: *And when I am ill, it is He who cures me* [3].

3.2 Sharing knowledge in Medical Fiqh issues and rukhsah ibadah

During hospital attachments and home visits, students are exposed to patients with various types of medical conditions. Some of the patients have acute diseases while others have chronic diseases. Some of the patients with chronic diseases are bed-ridden and unable to look after themselves. This is the oppurtunity for the students to explore the religious practice of the patients. Patients with diabetes mellitus on insulin therapy often experiencing hypoglycaemic attacks during fasting month because of not adjusting the dose and timing of the medication properly. Patients with bladder incontinence, on continous bladder drainage bag or on stoma bag have are in doubt when performing prayers *(solat)* and did not know that they must perform it with their conditions. Some of the patients that are immobilized due to injury or medical condition ignore their religious duty due to not having enough knowledge in performing prayers in lying or sitting positions. With proper guidance and discsussion with the conditions of the patients, the students can learn themselves and can share their knowledge with the patients.

3.3 Educational visits to selected institutions

During this posting, students are required to organised educational visits to several institutions. Among the institutions selected include clinic for the elderly in tampin, old folks home and special need children rehabilitation center.

Visits to Clinic for the elderly and old folks home is in line with the teachings of our prophet. Prophet Muhammad (peace and blessings be upon him), taught caring for the elderly irrespective of sex, color, or religion, and he himself set a great example in practicing the principles he taught. In a hadith narrated by Anas ibnMalik (may Allah be pleased with him), the Prophet (peace and blessings be upon him) said, *"If a young man honors an elderly on account of his age, Allah appoints someone to honor him in his old age (At-Tirmidhi)"* [4]. The Prophet also considered respecting the elderly is a way to show reverence for the Almighty. In another hadith, Abu Musa Al-Ash`ari (may Allah be pleased with him) narrated that the Prophet (peace and blessings be upon him) said, *"It is out of reverence to Allah to respect the white-headed (aged) Muslim (Abu Dawud)."* [5].

Besides the respecting the elderly, Islam also teaches us to be kind to the children because children are great blessing from Allah. Visit to special need children rehabilitation center enable the student to learn the needs of special children (with physical or mental disabilities), ways to approch them, participating in the rehabilitation activities, interact with their family members, determine their barriers to wellness and plan the best rehabilitation method to optimise their physical and mental functions. Being passionate in children was shown by the prophet

himself as reported by Anas ibn Malik (may Allah be pleased with him), the servant of the Prophet, had another recollection: *I never saw anyone who was more compassionate towards children than Allah's Messenger (peace and blessings be upon him). His son Ibrahim was in the care of a wet nurse in the hills around Madinah. He would go there, and we would go with him, and he would enter the house, pick up his son and kiss him, then come back* [6].

3.3 Effective Muslim Communication Skills

Effective communication skill is one of the important skills that are important to medical students. As future doctors, they must be able to communicate well with the patients and their families. As asserted by the Association of American Medical Colleges, "Physicians must be able to communicate with the patients and patients' families about all of their concerns regarding patients' health and well being". Talking to patients is not solely meant for understanding their thoughts and attitudes; it also serves as an avenue for students to learn about relationship that are appopriate between patients and students. Students are taught to approach a Muslim patient with *salam* and smile. Abu Hurairah (may Allah be pleased with him) narrated that the Prophet (peace and blessings be upon him) said, *"The young should (initiate) salutation to the old, the passerby should (initiate) salutation to the sitting one, and the small group of persons should (initiate) salutation to the large group of persons"* [7]. In that hadith, the Prophet (peace and blessings be upon him) gives practical examples of Islamic etiquette and starts with a token of respect to the old. Thus the young should take the initiative toward the aged in greeting and also helping, showing kindness, visiting, advising, phoning, and so on. Proper addressing of the elderly with the address *Encik*, *Tuan Haji*, *Puan* or *Hajah* is encouraged.

3.4 Community-based Scientific Research Project and Intervention Programme

Community-based study is a group scientific research project, which is carried out during the Community Health Posting. By doing the research, problems in the community is identified, studied, analyzed and discussed. Proper intenvention is planned and performed based on the study outcome. Dissemination of study results is done by presenting the result of the study in local and international conferences as well as in the medical journals.

Early Muslim scholars used to acquire an extensive knowledge, not only in Islamic and linguistic studies, but also in the field of medicine, chemistry, and natural sciences. Knowledge has to be based on evidence [8]. In the Holy Quran, Allah says: *...can there be another god besides Allah? Say bring forth your proof if you are telling the truth!* [9]. Based on the above, scientific research is considered by some scholars as *fardhu kifayah* (collective religious duty).

4. The outcome of Naqli and Aqli Integration in Community Health Posting Module

In general, the students have acquired Islamic knowledge through the integrating of *Naqli* and *Aqli* components with Islamic principles and values in the Community Health Posting. These were demonstrated by the use of certain selected hadiths and quranic verses in the case presentations, essays and case write-up of the students. During the presentation of community-based case study, they had discussed the study findings with Islamic perspectives.

5. Discussions

The components of *Naqli* and *Aqli* with Islamic principles and values had been succesfully integrated into the Community Health Posting since the beginning of the programme. The Islamic input in medical education provides both professionalism, with divine injunctions of perfection and competence, together with a holistic approach towards patients, family, society, disease, health, life and death [10]. The integration of Islamic principles and values in the medical curriculum represents a new paradigm shift in medical education that aims to inculcate ethical values in the students so that upon graduation, they would be a different 'breed' of doctors who not only excel in their professional duty but also portray conduct, (*akhlaq*) expected of a good Muslim [11]. Effective communication skills are also important in becoming a good Muslim doctor. Students are taught during this posting on making the five axial diagnosis which consist of physical, psychological, social and behavioural diagnosis as well as effect on functionaing of patients [12]. At the end of the posting, the students are able to describe the importance of comprehensive and holistic care in patient management [13]. By conducting community-based research, students familiarize themselves with common medical problem and health issues faced by the community. Throughout the planning and implementation period they are to work closely with the district's health officer and staff and with the village committee. They also have to recognize the role of political figures in the district. In short, their research activity will lead them to realize of who in the community should be involved in health and how can they be mobilized Like in other universities, major constraints of the Community Health Posting are time and funding [14]. The time given (4 weeks) to conduct the study often not enough for complete data collection. The constraint due to limited funds can hinder students from exploiting various strategies in the intervention activities.

6. Conclusion

In general, the students have acquired Islamic knowledge through the integrating of Naqli and Aqli components in the Community Health Posting. This had been demonstrated and reflected in their attitudes and overall behaviours that potray good Muslim profesionalism in handling patients, understanding of diseases and care of the sick.

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Expression of TLR-2 of Mice Infected by Mycobacterium Tuberculosis by Administration of Methanol Extract of Graftophyllum Pictum L. Griff

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Abstract - Tuberculosis is a chronic infectious disease caused by Mycobacterium tuberculosis. Toll-Like Receptor-2 (TLR-2) signaling plays a role in the body's defense system against Mycobacterium tuberculosis. Daun Ungu (Graptophyllum pictum L. Griff) is a traditional herbal plants which has medicinal properties for infectious and inflammatory diseases. This study aimed at analyzing the expression of TLR-2 of mice with the administration of methanol extract of Daun Ungu (Graptophyllum pictum L. Griff). The method used was an experimental laboratory, thirty male mice aged 8-12 weeks were randomly allocated into 5 groups; 2 control groups (K0, K1), 3 treatment groups (P1, P2, P3). K1, P1, P2 and P3 were infected using Mycobacterium tuberculosis at a dose of 108 ml and left for 4 weeks. At the fifth week groups P1, P2 and P3 were administered with methanol extract of Graptophyllum pictum L. Griff (EMDU) at a dose 1,703 mg, 3.406 mg, 6.812 mg. The administration of 0.2 ml was conducted every other day for 2 weeks. Examination of the expression of TLR-2 was carried out using immunohistochemical techniques. The data were analyzed using ANOVA and correlation analysis. The results showed that the EMDU in mice infected with Mycobacterium tuberculosis by immunohistochemical technique was succeed that the EMDU could increase the expression of TLR-2. The results of the correlation test showed a positive correlation between dose EMDU with TLR-2 expression. The conclusion of this study is that the EMDU can increase expression of TLR-2.

Keywords : TLR-2, Mycobacterium tuberculosis, EMDU.

1. Introduction

World Health Organization (WHO) states that there are 22 countries with high prevalence of tuberculosis. Most patients are in Asia (55%) followed by Africa (30%), Middle East (7%), Europe (4%) and America (3%). Thus, most parts of the world are still not free from tuberculosis. In Indonesia, the incidence of tuberculosis is the fifth rank in the world, after India, China, South Africa and Nigeria. According to the *Household Health s*urvey held by the Ministry of Health in 2001 that every year 583,000 new tuberculosis patients have been found of which 50% are patients with the category of positive *Acid-Fast Bacillus* (AFB) meant as transmitters [1],[2].

Tuberculosis is an infectious disease that is highly dependent on the immune response. Severity of tuberculosis disease is mainly influenced by the host immune response. Various theories state that innate immunity is a leading immune response, particularly the most potent macrophages against *Mycobacterium tuberculosis*. Macrophages are professional phagocytic cells with main function to destroy immunogen and as *Antigen Precenting Cells* (APC) through several receptors recognizing microbes associated with its function to stimulate cell migration to the site of infection and stimulate the production of microbial substances. Pathogen recognition accuracy is affected by receptor that influences the attachment of pathogens on APC. Allegedly receptor that activates macrophages to stimulate innate immunity one of which is the *Toll-Like Receptor* (TLR) that will affect the transcription factors of *Nuclear Factor Kappa Betha* (NFkB), then stimulates the phagocytic activity and cytokine production. One of the 10 TLRs, TLR-2, is considered to be the key mediator in innate immunity against *Mycobacterium tuberculosis*. In tuberculosis, it is assumed to occur the suppression of TLR-2 which results in a decrease in the phagocytic function and affects the proinflammatory cytokines TNF-α and IFN-γ and anti-inflammatory cytokine TGF-β. Changes in cytokine levels affect the level of damage to lung tissue [3],[4].

Daun ungu (*Graptophyllum pictum*) is one of Indonesian traditional medicinal plants. It is included in the list of 66 medicinal crops established by the Decree of the Minister of Agriculture No. 511 / Kpts / PD.310 / 9/2006. Indonesian people use this plant to treat swelling, ulcers, hemorrhoids and to help menstruation process [5], and is also used to cure bleeding cough [6]. Tuberculosis is a chronic infectious disease that of which bleeding cough is one of the clinical symptoms. Several studies have been conducted to mention that the ethanol extract of *Daun*

Ungu (Graptophyllum pictum (L) Griff) has activity against *Mycobacterium tuberculosis H37Rv* antimikobakterial *in vitro* [7].

Based on the data from scientific reports about the capabilities of the *phytopharmaca* activity of *Daun Ungu* and its long term use in the midst of society without causing any side effects, thus the researchers want to observe further about whether the methanol extract of *Daun Ungu* affects the expression of TLR-2 in the lung tissue of mice infected with *Mycobacterium tuberculosis*. The goal of this study is to analyze the increased expression of TLR-2 in lung tissue of mice infected with *Mycobacterium tuberculosis* by administration of methanol extract of *Daun Ungu* (EMDU).

2. Methods

This study was an experimental study in mice (Mus muculus) Balb type / c to compare the groups of mice infected with *Mycobacterium tuberculosis* (M.tb), mice infected with M.tb accompaned with *Daun Ungu* methanol extract (EMDU) at a dose of 1,703 mg / kg / BW), 3.406 mg / kg / BW), and 6,812 mg / kg / WB) for 2 weeks, and the group of mice without any treatment (normal).Tuberculosis infection in animal models is due to the exposure (injection) of 60µL *of Mycobacterium tuberculosis H37Rv* with 10^8 per mL concentration by means of intra-tracheal [8]. TLR-2 protein expression was the expression of TLR-2 protein derived from the lung tissue of mice, examined using immunohistochemistry examination using monoclonal antibodies anti-TLR-2. Calculations performed on immunoreactive cells showed positive expression and appeared reddish-brown in the cytoplasm. Counted as many as 10 fields of views using a light microscope at a magnification of 400 times to take average value. Immunoreactive cell count average value entered as data [9],[10].

Preparation of Test Materials : *Griff L. pictum Graptophyllum* leaves taken from Traditional Medicine (OBTRA) Garden of Traditional Medicine Division of the Center of Health Development and Research (PUSLITBANGKES) Surabaya. The plant has been determined in Indonesian Institute of Sciences Plant Conservation Unit *Kebun Raya Purwodadi, Pasuruan*. Making EMDU was conducted by maceration using 80% of methanol. To determine the class of compounds contained in *Daun Ungu* phytochemical screening was carried out using *Thin Layer Chromatography* (TLC) method [11], [12]. Dose calculation based on the dose immunomodulatory of *Daun Ungu* was 0.2 ml infusion 10% /head / po / day [13].

Analysis of Data. Sequence analysis of the data: the Kolmogorov-Smirnov Normality Test: to determine the normal distribution of data (p> 0.05). Levene test conducted to determine the homogeneity of the data. If the variance of the data was homogeneous (p> 0.05) followed with ANOVA test and Duncan's test orderly. Path analysis was conducted to determine the correlation.

3. Results and Analysis of Research

Table of the mean, standard deviations and ANOVA test TLR-2 expression in lung tissue of mice.

Groups	Mean	Standard Deviation	Anova
Control (-)	$4{,}083^{a}$	1,59	F =38.57
Control (+)	$6{,}00^{a,b}$	0,71	p= 0,000
P1 / (*M.tb*+D1)	$7{,}83^{b}$	1,69	
P2 / (*M. tb*+D2)	$15{,}17^{c}$	3,28	
P3 / (*M. tb*+D3)	$15{,}42^{c}$	2,27	

Note : varied *superscript* shows the present of significant differences (p<0,05)

Test Results of ANOVA shows a significant difference (p = 0.000). This means that the increased expression of TLR-2 is caused by EMDU administration. The results of the Duncan test demonstrate that expression of TLR-2 increased along with increasing dose EMDU but increased expression of TLR-2 seems real (significant) in the P2 group (dose of 3.406 mg / kg) and P3 (dose of 6,812 mg / kg) when compared to control (+). Correlation between EMDU with TLR-2 is positive and significant (r = 1.487, p = 0.000). This means that the higher the dose EMDU will be followed by the higher expression of TLR-2.

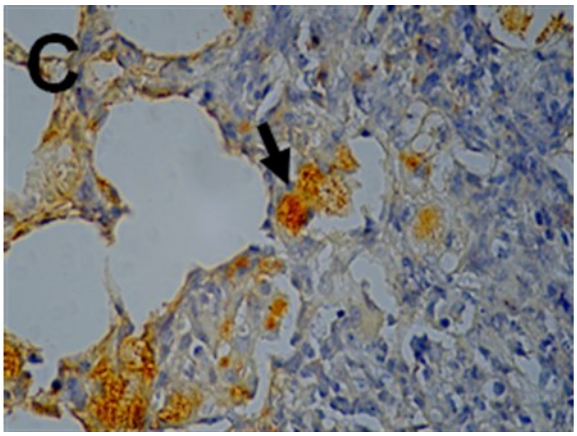

Figure 2.1 Expression of of TLR-2 lung tissue of mice infected with *Mycobacterium tuberculosis* and treated with EMDU. Brown macrophage cytoplasm (mark →) indicates a positive result. Immunohistochemical staining. 400X magnification.

4. Discussion

The results of this study demostrates that the methanol extract of *Daun Ungu* (EMDU) may

increase the expression of TLR-2 in groups P1, P2 and P3, likely due to the effects of flavonoids of EMDU which enhance the activity of immunomodulating on alveolar macrophage cell with co-stimulation of *Mycobacterium tuberculosis* leading to increased TLR expression-2.This mechanism occurs due to *Daun Ungu*'s flavonoids. Flavonoids identified from the methanol extract of *Daun Ungu* (EMDU) i.e. *quercetin, kaempherol* and *myrecetin flavonols* [6], [13]. *Kaempherol flavonol* is able to bind to the estrogen receptor (ER) on the surface of macrophages [14]. Therefore, administering EMDU is expected to result bonds between flavonols and estrogen receptors on macrophages. It will result in a transduction signal delivery. It is known that the *quercetin flavonol* can activate TLR-2 and NF-kβ [15], [16]. Increased activation of the NF-kβ will increase the TLR-2 gene transcription as well. Expression of TLR-2 increased with increasing dose EMDU. This is consistent with the results of the research conducted by Kiat Lim *et al*., (2013), that the *flavonoid quercetin* is able to increase the production of cytokines IL-1β through increased expression of TLR-2 on monocytes [15]. Based on Duncan test, increased expression of TLR-2 in this study is evident (significant) in the positive group (K1) with P2 group (dose 3.406 mg / kg/BW) and P3 (dose of 6,812 mg / kg/BW). It shows that EMDU is able to increase the expression of TLR-2 in mice infected with *Mycobacterium tuberculosis* significantly at dose 2 (3.406 mg / kg/BW) and dose 3 (6,812 mg / kg/BW), whereas at dose 1 (1.703 mg / kg/BW) increased expression of TLR-2 is not significant. This likely occurs because EMDU at dose 1 has low affinity. Affinity is a measure of the ability of drugs to bind to the receptor [17], [18]. Due to the low EMDU quantity of dose 1, the ability to provide therapeutic effects has not been optimized so that the expression of TLR-2 is not increased significantly.

The increased expression of TLR-2 becomes advantageous. The TLR-2 in patients with tuberculosis serves to increase the maturation of dendritic cells to enhance the ability to perform phagocytosis against *Mycobacterium tuberculosis* [19], [20]. TLR-2 has a role as introductory mediator of *Mycobacterium tuberculosis* and initiates innate immune response against infectious disease. *Mycobacterium tuberculosis* recognized by TLR-2 will activate macrophages and dendritic cells. According to research from Takeda and Akira (2004), the expression of TLR-2 will lead to immature dendritic cells located in the periphery becomes mature and subsequently heads to limfonoduli, it will initiate an adaptive response activation which then produces a signal for transcription of NF-kβ which is the main regulators of the immune response [21]. Based on the results, it can be concluded that the methanol extract of the *Graftophyllum pictum L. Griff* leaves is able to increase the expression of TLR-2 in lung tissue of mice infected with *Mycobacterium tuberculosis*.

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Profiling of Fatty Acids in Lard during Frying Using GC-FID and Chemometrics

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Abstract - The composition of Fatty Acids (FAs) in lard during frying at different temperatures (120 ^{0}C, 180 ^{0}C, 240 ^{0}C) and times (0.5 hr, 1hr, 2hrs, 3 hrs) was determined in the form of Fatty Acid Methyl Esters (FAME) using Gas Chromatography-Flame Ionization Detector (GC-FID). Experiments were performed using different percentages of lard (0 %, 1 %, 5 %, 10 %, and 20 %) in refined palm cooking oil. A multivariate data of FAME from the chromatograms was analyzed using Principal Components Analysis (PCA) in which the scores and loadings plots from the PCA were studied to find how the profiles of FAs in lard and the cooking oil were affected by the variables.

1. Introduction

Lard is an edible fat derived from pig derivatives and is used as an important ingredient for cooking in some countries. Lard is deliberately added into oil and edible oil to reduce production cost or for the better taste to any food. However, as the largest Muslim community in Malaysia and from religious views, any parts of pigs are not allowed to be consumed or *non halal*. Therefore, there is a requirement to develop analytical methods to identify lard especially in cooking oil.

The detection of adulteration of used cooking oil by non- *halal* substances such lard is a very difficult task since the FAs composition of animals and plants are similar. The complexity caused by degrading of the chemical structures during frying at high temperatures. Gas Chromatography flame-ionization (GC-FID) has been used to identify FAs compositions in edible fats and oils [1]. This analytical instrumental produces high dimensional multivariate data and required statistical tools for data interpretation.

Nowadays, the development of computers makes possible to process large multivariate data with statistical and chemometrics softwares. Principal Components Analysis (PCA) is an application of chemometrics used as a tool of exploratory analysis to reduce large complex data sets or rearranges the data to exploit linear structure. The transformation of new data must have correlation between the new variable that called as Principal Components (PC). PC is known as latent variable and eigenvector. Results of a PCA are usually discussed by viewing graphical of component scores called scores plot and loadings plot.

PCA have ben often used for the classification and comparison of different edible fats and oils. Some examples recent studies are, profiling FAs of lard using comprehensive Gas Chromatography hyphenated with Time of Flight Mass Spectrometry (GC-ToF) [2.3], the differentiation of lard from other animal fats in admixtures of some vegetable oils using Liquid Chromatographic (LC) data coupled with multivariate data analysis [4].

Although lard detection has been reported using PCA technique, the frying of lard in cooking oils has yet to be done. Here we studied the profile changes of FAs in three parameters; (i) percentage of lard (ii) temperature of heating and (ii) time of frying. Palm oil was selected in this study due to the most favorite cooking oil used in Malaysia.

2. Material And Method

2.1 Chemicals and Reagents

The analytical solvents used for fat extraction and GC analysis were methanol 99.9 %, acetone 99.9 %, n-hexane 99.9 %, chloroform 99.9 % and sodium methoxide 1% solution. All mentioned solvents were purchased from Sigma Aldrich, USA.

2.2 Fatty Acids Standard

The standards FAME Mix was purchased from Supelco, Sigma–Aldrich, USA.
Abbreviations of 25 FAs for carbon atom and IUPAC names are; 1; Lauric (C12:0), 2; Myristic (C14:0), 3; Palmitic(C16:0) 4; Octadecenoic (C18:1), 5; Linoleic Acid (C18:2), 6; Eicosonic (C20:1), 7; Margaric (C17:0), 8; Behenic (C22:0), 9; Docosatetraenoioc (C22:4), 10; Caproic (C6:0), 11; Capric (C10:0), 12; Tridecanoic (C13:0), 13; Stearic (C18:0), 14; Docosapentaenoic C22.5), 15; Tricosanoic (C23:0), 16; Nervonoic (C24:1), 17; Butyric (C4:0), 18; (C22:6) Docosahexanoic, 19; Arachidic(C20:0) , 20; Caprylic (C8:0), 21; gamma-Linolenic (C18:3n6), 22; gamma-Eicosatrienoic (C20:3), 23; Docosadienoic

(C22:2), Docosahexaenoic (C24:0) and 25; Arachidonic (C20:4).

2.3 Samples Preparation

Lard (rendered from the belly pork) and palm oil were purchased from a local supermarket. Lard was heated at 90^{0}C for 15 min to melt and then blended with palm Olean. The fat was extracted following to the Bligh-Dryer method (1959) [5] with slight modification. The samples of spiked lard (0 %, 1 %, 5 %, 10 % and 20 %) in palm oil were prepared and heated on a digital hot plate at selected temperatures (120 ^{0}C, 180 ^{0}C and 240 ^{0}C) at interval times (0.5, 1, 2, 3 hrs). The summary and its labeling are as in Table 1.

Table 1: Summary of samples treatment and labeling.

T ^{0}C	Frying times (hrs)	Percentage of lard in cooking oils 0 %	1 %	5 %	10 %	20 %
120	0.5	*a1*	*b1*	*c1*	*d1*	*e1*
	1	*a2*	*b2*	*c2*	*d2*	*e2*
	2	*a3*	*b3*	*c3*	*d3*	*e3*
	3	*a4*	*b4*	*c4*	*d4*	*e4*
180	0.5	*a5*	*b5*	*c5*	*d5*	*e5*
	1	*a6*	*b6*	*c6*	*d6*	*e6*
	2	*a7*	*b7*	*c7*	*d7*	*e7*
	3	*a8*	*b8*	*c8*	*d8*	*e8*
240	0.5	*a9*	*b9*	*c9*	*d9*	*e9*
	1	*a10*	*b10*	*c10*	*d10*	*e10*
	2	*a11*	*b11*	*c11*	*d11*	*e11*
	3	*a12*	*b12*	*c12*	*d12*	*e12*

2.4 Analysis of Fatty Acid Methyl Esters (FAME) in Samples Using GC-FID

FAs composition was determined by conversion of fatty acid methyl esters (FAME) following to the method of Cocks and Van Rede (1966) with a slight modification [6]. FAME was prepared by adding 950 µL of n-hexane into 50 mg of fat followed by 5 µL sodium methoxide. The mixtures were vortexed for 5s and allowed to settle for 40 min. A volume of 1µL was collected from the top layer and injected into a gas chromatography.

Gas chromatography-flame ionization detector (Agilent 7980) was fixed with a polar capillary column HP 88 with specifications 100m, 25 mm and 25 µm film. The temperature of the column was set up at 100 ^{0}C (for 4 min), increased to 240 ^{0}C at 3 ^{0}C/min (for 15 min). The temperature of the injector was set up at 225 ^{0}C and detector at 285 ^{0}C. The run time was set up 60 min for a sample [7]. The FAME peaks were identified by comparing their retention time with certified reference standards of FAME. Percentage of FAs was calculated based on the peak area of a fatty acid species to the total peak of all the fatty acids in the oil samples.

2.4 Data Analysis

A total of 60 x 25 matrix of spiked lards and FAs were normalized using the maximum FA values in each sample before subjected to PCA. PCA was performed by using Unscrambler software (X10). The multivariate data set of FAME were subjected to PCA in order to reduce the data set to scores and loadings matrices.

3. Results and Discussion

3.1 Fatty Acids Composition

The composition of FAs in the samples are as shown in Table 2. (Appendix). Results of FAs composition in cooking oils (blank) were corresponded to results of the others studies in fried cooking oils or used cooking oil [8][9].

3.2 PCA Analysis

PCA has been performed on data set samples. PC which has eigenvalues greater than 1 was chosen to reduce the data dimensionality as shown in Table 3. First three PCs of total variability was explained PC1 (95%), PC2 (3%) and PC 3 (1%).

Table 3: Scores and plots of PC1, PC2 and PC3

	% Variance PC1	PC2	PC3
Scores	95	4	1
Loadings	95	3	1

PCA of scores and loadings plot in Figures 1 and 2, visualised to discriminate the different types of FAs that significantly contributes to PCA model.

PC 1 describes 95 % of the variances in the data set and its loadings indicate that it has high contributions from FA 4; Octadecenoic (C18:1) and FA 5; Linoleic Acid (C18:2) at negative loadings. PC 2 showed positive loading for FA 3; Palmitic(C16:0) . Specific clusters of correlation between variables can be visualized by comparing loading plot between PC1 and PC2 as shown in Figure 1 and Figure 2. Figure 1 depicts the score plots of FA in samples generated from comparing PC1 and PC2 revealed 5 district sample groupings.

Cluster I showed spiked lard 1-5 % with temperatures ≤ 180 ^{0}C with times 0.5, 1, 2 and 3 hours. This cluster also explained to 1-5 % spiked of lard with temperatures 240 ^{0}C with temperatures 0.5 hr. Cluster II 0 % with temperatures 120 ^{0}C (all times), 180 & 240 ^{0}C with 1 hr heating time. The mixing percentages lard 0-20 % with temperatures ≤ 180 ^{0}C with frying time 0.5-2hrs.

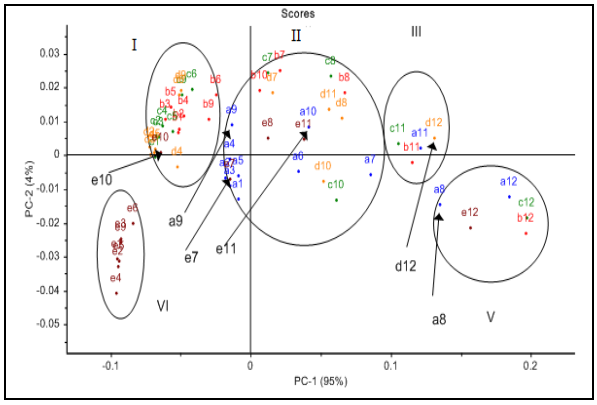

Figure 1: scores plot of PC1 and PC2

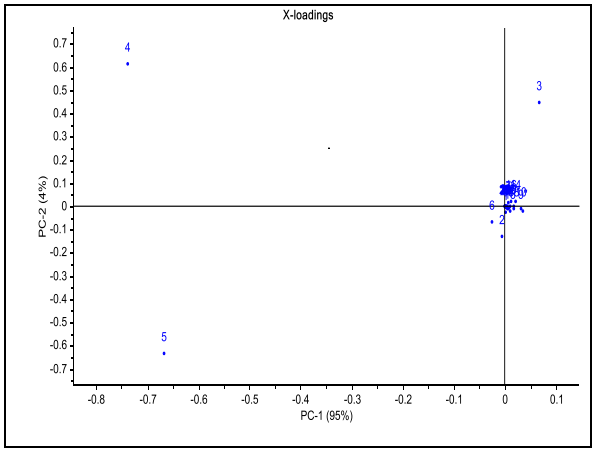

Figure 2: Loadings plot of PC1 and PC2.

Cluster III showed spiked lard 0 %-5 % with temperatures at 240 ^{0}C with 2 hr indicated that high temperatures with longer heating time started grouping differently. Cluster IV (0-20 %) with high temperatures 240 ^{0}C maximum heating time 3 hr. At this points samples contained lard and blank not showed the differences. Cluster V showed the grouped of lard with temperatures ≤ 180 ^{0}C at temperatures less than 1 hr. The points of samples that indicated by arrow are not supposedly in the groups. This may in different groups and may cause by the error during FAME process. Replication need to do to check this error. Figure 3D of PCA will give the better result to the scores and loadings plots.

Scores and loadings of first three PC can be plotted as a scatter diagram. For better visualization the scatters diagram were rotated as in Figure 3 and Figure 4. Five clusters were spotted to differentiate classes among the blanks and cooking oils containing lard. There are two samples different of samples point at 3D diagram showed by the arrow. It can be seen at sample *a9* grouped at same cluster I with *b9*, *c9*, *d9* and *e8*. However *a7* are grouped to the others. The others error at diagram 2D are remains and unchanged as indicate in Table 4. The greater temperatures produced more significant differentiation of PCA model. High frying temperature accelerates thermal oxidation and polymerization of oils [10]. Exceptional parameters can be seen temperatures 240 ^{o}C has same grouping with temperatures < 180 ^{o}C at frying time 0.5 hr.

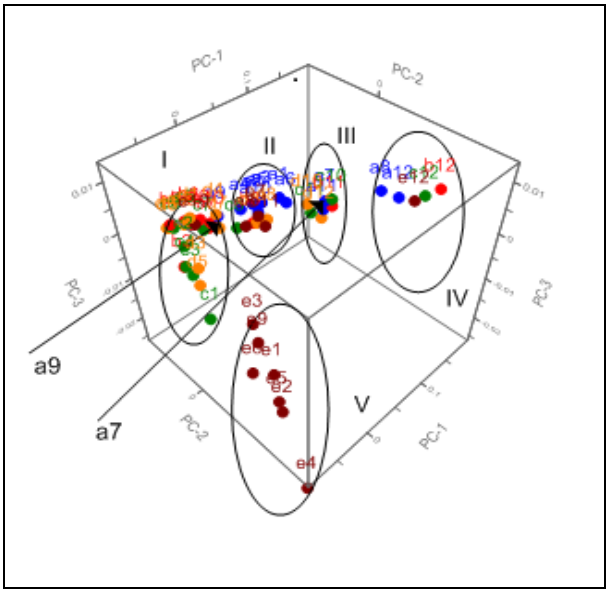

Figure 3: Scores plot of normalized data in 3D rotation.

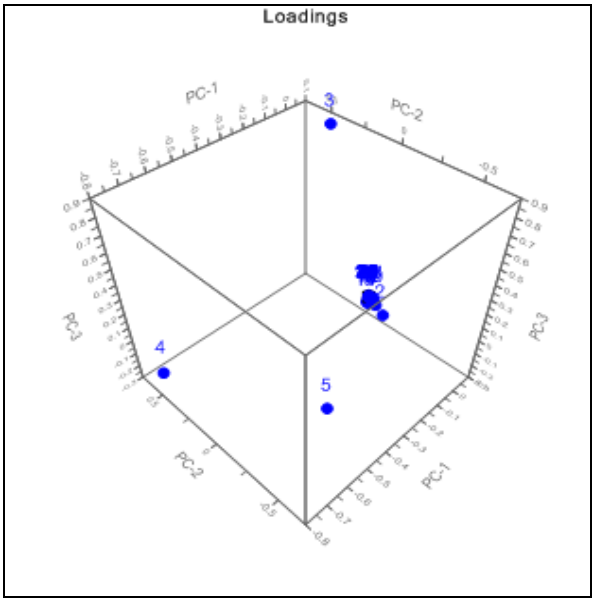

Figure 4:Loadings plot of normalized data in 3D rotation.

These variables described the groups for each clusters; cluster I (FAs 4; Octadecenoic (C18:1)), clusters II, III (FAs 3; Palmitic(C16:0)), clusters IV, (FAs 2, 3, 6 & other FAs) and cluster V (FAs 5; Linoleic Acid (C18:2)). Cluster V are well separated from the other clusters and are significantly different. Cooking time also influenced the PCA model and this

can be seen at clusters IV and V. The comparisons of 2 type graph, 2D and 3D, it can be concluded that blanks and cooking oils containing lard were insignificantly different at temperatures >240 °C at 1, 2, 3 hrs cooking time for all concentrations. The samples contains 20 % lard are significantly different at lower temperatures and times. Frying time increases the contents of free fatty acids and polar compounds such as triacylglycerol dimers and oxidized triacylglycerol's [11].

Table 4: Scores distribution at 2D diagram of PC1 X PC 2

Clusters/ Groups	I	II	III	IV	VI
Labels	b1-b6, c1-c6, d1-d6	a1-a5	a11, b11, c11	a12,b12,c12, e12	e1-e6
	b9, c9, d9	a9*	d12*	a8*	e9
	e10*	b8, c8, d8, e8			
		a7, b7, c7, d7, e7			
		a 10, b10, c10, d10			
		e11, d11*			
Definitions	1%- 120°C -0.5, 1, 2 & 3hrs.	0%-120°C: 0.5, 1, 2, 3hrs.	0%, 1% & 5%-240°C; 2 hrs.	0%, 1%, 5% & 20%-240°C;3hrs	20% , 120°C; all time, 180°C; 0.5 &1 hrs
	5%- 120°C -0.5, 1, 2 & 3hrs	1%-20%,180°C;2, 3 hr s	*except 10%-240 °C, 3 hrs	*except 0%-180°C- 3 hrs	20% , 240°C, 0.5hr
	10%- 120°C -0.5, 1, 2 & 3hrs.	0%- 20%- 180°C; 1hrs.			
	1%, 5% & 10%-180; 0.5hr & 1hrs.	0%-20% - 240°C, 1hrs.			
	1%, 5%, & 10%-240°C - 0.5hr.	*except 20%, 240°C -2hrs, 0%, 240°C -0.5hr, 10%, 240°C - 3hrs.			
	*except 20%, 240°C; 1hrs.				

**3D diagram also showed same results except the location of samples a9 and a7.

4. Summary

The combination of experimental GC-FID and FAs data along with chemometrics approach such as PCA can be employed in multivariate data for differentiation of cooking oil spiked with 20 % lard. Three FAs: 3; Palmitic(C16:0) 4; Octadecenoic (C18:1) and 5; Linoleic Acid (C18:2) are significantly observed in the frying tests. Replication samples are suggested to be done to check the esterification process is completely performed.

5. Acknowledgments

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Appendix

Table 2: Fatty Acids Composition in 60 samples identified by GC-FID.

Samples	FAs																										
	1	2	2"	3	4	5	6	7	7"	8	9	9"	10	11	12	13	14	14"	15	16	17	18	19	20	21	22	23	24	25
a1	0.39	1.34	0	45.39	42.75	10	0.14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
a2	0.37	1.31	0	45.14	43.03	10.01	0.14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
a3	0.38	1.32	0	45.22	42.93	10.01	0.15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
a4	0.37	1.31	0	45.23	43.21	9.73	0.13	0	0.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
a5	0.38	1.31	0	45.39	43	9.76	0.14	0	0.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
a6	0.4	1.37	0	46.6	42.75	8.56	0.12	0	0.04	0.01	0.04	0	0.08	0.03	0.01	0	0	0	0	0	0	0	0	0	0	0	0	0	0
a7	0.41	1.42	0	47.96	42.17	7.13	0	0.08	0.05	0.01	0.17	0	0.15	0.04	0.01	0.01	0.16	0.01	0.22	0	0	0	0	0	0	0	0	0	0
a8	0.4	1.42	0	49.45	41.41	6.03	0	0.11	0.1	0.02	0.29	0	0.2	0	0	0.02	0.23	0.04	0.29	0	0	0	0	0	0	0	0	0	0
a9	0.37	1.29	0	45.37	43.63	9.32	0	0.03	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
a10	0.41	1.41	0	48.87	42.03	5.74	0	0.03	0.08	0.02	0.12	0.33	0.26	0.05	0	0.02	0.26	0.02	0.28	0.09	0	0	0	0	0	0	0	0	0
a11	0.42	1.43	0	48.61	41.88	6.09	0	0.03	0.08	0.03	0.11	0.33	0.25	0.05	0.02	0.21	0.05	0.02	0.11	0.08	0.2	0	0	0	0	0	0	0	0
a12	0.46	1.52	0	50.65	40.73	4.31	0	0.05	0.09	0.03	0.21	0.54	0.42	0.06	0.03	0.38	0.03	0.02	0.11	0.16	0.2	0	0	0	0	0	0	0	0
b1	0.36	1.28	0	44.16	43.63	10.33	0.24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
b2	0.36	1.28	0	44.22	43.69	10.28	0.17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
b3	0.35	1.24	0	43.78	44.15	10.32	0.17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
b4	0.36	1.28	0	44.31	43.81	10.06	0.17	0.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
b5	0.35	1.26	0	44.07	43.94	10.19	0.17	0.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
b6	0.36	1.29	0	44.95	43.91	9.25	0.15	0.03	0	0	0	0.06	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
b7	0.37	1.31	0	46.05	43.72	7.75	0	0.05	0	0.02	0.17	0	0.12	0	0	0	0.16	0	0.01	0.27	0	0	0	0	0	0	0	0	0
b8	0.38	1.35	0	47.19	43.01	6.68	0	0.11	0	0.02	0.09	0.32	0.18	0	0	0.02	0.29	0	0.02	0.16	0.19	0.01	0	0	0	0	0	0	0
b9	0.37	1.28	0	44.78	43.67	9.64	0.15	0.03	0	0.01	0	0	0.07	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
b10	0.37	1.3	0	45.86	43.8	8.4	0	0.08	0	0.01	0.09	0	0.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
b11	0.42	1.43	0	48.61	41.88	6.09	0	0.03	0.08	0.03	0.11	0.33	0.25	0.05	0.02	0.21	0.05	0	0.02	0.11	0.08	0.2	0	0	0	0	0	0	0
b12	0.47	1.54	0.01	50.88	40.05	4.36	0	0.05	0.11	0.04	0.23	0.57	0.44	0.06	0.04	0.36	0.1	0	0.04	0.14	0.15	0.26	0	0.04	0.01	0.02	0.02	0	0
c1	0.35	1.29	0	43.28	44.14	10.74	0.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
c2	0.37	1.32	0	43.62	43.82	10.68	0.21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
c3	0.36	1.3	0	43.55	43.99	10.59	0.2	0.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
c4	0.37	1.31	0	43.66	44.02	10.43	0.19	0.03	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
c5	0.37	1.33	0	44.03	43.64	10.41	0.2	0.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
c6	0.37	1.33	0	44.41	44	9.62	0.17	0.04	0	0	0	0.06	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
c7	0.38	1.36	0	45.71	43.67	7.99	0	0.06	0	0.02	0.23	0	0.13	0	0	0	0.17	0	0.02	0.27	0	0	0	0	0	0	0	0	0
c8	0.39	1.39	0	46.81	43.17	6.76	0	0.12	0	0.03	0.1	0.35	0.2	0	0	0	0.32	0	0.02	0.15	0	0.21	0	0	0	0	0	0	0
c9	0.37	1.31	0	44.25	43.99	9.87	0	0.17	0.03	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
c10	0.44	1.5	0	47.02	41.94	8.1	0	0.13	0.09	0.03	0.07	0.14	0.16	0.04	0	0.01	0.13	0	0.02	0.06	0	0.13	0	0	0	0	0	0	0
c11	0.43	1.49	0	48.21	42.07	6.14	0	0.04	0.1	0.03	0.14	0.33	0.27	0.05	0	0.03	0.21	0.05	0.03	0.09	0.09	0.22	0	0	0	0	0	0	0
c12	0.48	1.62	0.01	50.8	40.15	4.14	0	0.06	0.13	0.04	0.25	0.57	0.48	0.07	0	0.05	0.34	0.08	0.04	0.13	0.18	0.3	0	0.05	0.02	0.02	0	0	0
d1	0.39	1.37	0	43.63	43.46	10.93	0.23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
d2	0.38	1.37	0	43.52	43.55	10.95	0.23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
d3	0.38	1.37	0	43.37	43.75	10.9	0.23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
d4	0.41	1.44	0	44.01	43.17	10.66	0.22	0.02	0	0.01	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
d5	0.36	1.35	0	43.4	43.92	10.74	0.23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
d6	0.37	1.37	0	44.17	43.99	9.88	0.2	0.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
d7	0.41	1.46	0	45.66	43.32	8.1	0	0.1	0	0.02	0.06	0.19	0.13	0.04	0.01	0	0.19	0	0	0.02	0.27	0	0	0	0	0	0	0.02	0
d8	0.43	1.53	0	46.94	42.6	7	0	0.04	0.07	0.03	0	0	0.2	0.04	0.02	0	0.33	0.25	0	0.02	0.16	0.19	0	0	0.01	0	0	0	0
d9	0.37	1.32	0	44.22	44.05	9.83	0.18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
d10	0.43	1.48	0	46.81	42.25	8.11	0.15	0.08	0	0.01	0.05	0.13	0.15	0.04	0	0.01	0.1	0.03	0	0.06	0	0.12	0	0	0	0	0	0	0
d11	0.43	1.52	0	46.7	42.79	7.15	0	0.04	0.08	0.03	0.1	0.28	0.22	0.05	0	0.02	0.2	0.05	0.02	0.09	0	0.23	0	0	0	0	0	0	0
d12	0.46	1.6	0	48.64	41.63	5.24	0	0.07	0.11	0.04	0.2	0.5	0.37	0.06	0	0.04	0.31	0.08	0.03	0.11	0.12	0.34	0	0	0	0	0	0	0
e1	0.41	1.62	0	42.09	43.27	12.26	0.31	0	0	0	0	0	0.04	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
e2	0.4	1.59	0	41.96	43.5	12.24	0.31	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
e3	0.42	1.64	0	42.37	43.15	12.09	0.3	0	0	0	0	0	0.04	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
e4	0.41	1.62	0	41.49	43.82	12.33	0.32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
e5	0.4	1.6	0	42.02	43.47	12.17	0.31	0	0	0	0	0	0.04	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
e6	0.4	1.6	0	42.42	43.67	11.61	0.28	0.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
e7	0.44	1.76	0	44.7	42.55	9.77	0.21	0.08	0	0	0.04	0.1	0.1	0.05	0	0	0.09	0	0	0.11	0	0	0	0	0	0	0	0	0
e8	0.44	1.74	0	45.23	42.59	8.58	0.18	0.11	0	0.03	0.06	0.23	0.16	0.05	0	0	0.21	0.06	0.02	0.31	0	0	0	0	0	0	0	0.01	0
e9	0.43	1.7	0	42.27	43.19	12.07	0.29	0.02	0	0	0	0	0	0.04	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
e10	0.45	1.74	0	43.45	43.17	10.71	0.23	0.04	0	0.03	0.05	0	0.1	0.04	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
e11	0.49	1.85	0	45.88	42.36	7.9	0.06	0.09	0	0.04	0.12	0.3	0.24	0.06	0	0	0.28	0	0.02	0.09	0	0.23	0	0	0	0	0	0	0
e12	0.57	2.09	0.01	49.17	40.23	5.45	0.09	0.11	0	0.04	0.19	0.46	0.45	0.09	0	0.03	0.27	0.05	0.03	0.09	0.15	0.31	0.01	0.05	0	0	0.02	0	0.03

Abbreviations of samples labeling;
Percentage of lard: a = **0%**, b = **1%**, c = **5%**, d = **10%** & e = **20%**. Temperatures of frying ^{0}C: 4-7= **120 ^{0} C**, 8-11=**180 ^{0} C** & 12-15=**240 ^{0} C**.. Time of frying in minutes: 4, 8, 12 = **0.5 hour**, 5, 9, 13= **1 hours**, 6, 10, 14= **2 hours**, 7, 11, 15= **3 hours**

Abbreviations of FAs atom carbon and IUPAC names;
1; Lauric (C12:0), 2; Myristic (C14:0), 3; Palmitic(C16:0) 4; Octadecenoic (C18:1), 5; Linoleic Acid (C18:2), 6; Eicosonic (C20:1), 7; Margaric (C17:0), 8; Behenic (C22:0), 9; Docosatetraenoioc (C22:4), 10; Caproic (C6:0), 11; Capric (C10:0), 12; Tridecanoic (C13:0), 13; Stearic (C18:0), 14; Docosapentaenoic C22.5), 15; Tricosanoic (C23:0), 16; Nervonoic (C24:1), 17; Butyric (C4:0), 18; (C22:6) Docosahexanoic, 19; Arachidic(C20:0) , 20; Caprylic (C8:0), 21; gamma-Linolenic (C18:3n6), 22; gamma-Eicosatrienoic (C20:3), 23; Docosadienoic (C22:2), Docosahexaenoic (C24:0), 25; Arachidonic (C20:4). (FA''means differents isomer but same FAs).

The Antioxidant Activity of Extracts From The Leaves of *Sonneratia Alba* J. Smith (1816)

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Abstract **-** Mangrove species are promising sources of natural antioxidants. *Sonneratia alba* leaf extracts were evaluated for their antioxidant activity using DPPH free radical scavenging assay. Maceration was made separately using petroleum ether and 95% ethanol. Only the ethanol-free extract exhibited positive antioxidant activity with the color change of DPPH solution and a decrease in absorbance relative to the negative control. P–level of 0.3498 ($p>0.01$) for ethanol-free extract vs positive control Phytochemical screening identified the presence of alkaloids, flavonoids, saponins, tannins and terpenoids. Further purification with column chromatography and DPPH assay revealed that the fraction 6, 7, 8 and 9 with positive antioxidant activity. P-level of 0.5011 ($p>0.01$) for bioactive fraction vs positive control. From FT-IR and GC-MS analysis, the bioactive fraction is a mixture of compounds, seven of which were reported having antioxidant activity. The synergistic combination of the phenolic compounds present may be responsible for the positive antioxidant activity.

Keywords: Antioxidant activity, *Sonneratia alba,* DPPH free radical scavenging assay, Color change, Absorbance.

1. Introduction

In the Philippines, heart disease and stroke are the leading causes of death with 120,000 Filipinos dying each year. Researchers have identified the risk factors involved in the development of cardiovascular disease. One such novel risk factor is the presence of reactive oxygen species (ROS) in the body. Many such reactive species are free radicals. When there is an imbalance between the generation of ROS and the activity of the body's antioxidant defences, this can lead to a condition known as oxidative stress. Severe oxidative stress can cause cell damage and death [1]. Oxidative stress is one of the major mechanisms in aging and age-related disease including cardiovascular disease. Antioxidants can terminate or retard oxidation process by scavenging free radicals [2]. Antioxidants could then be used as an inexpensive means of prevention and possibly, treatment of various oxidative stress associated disease including cardiovascular disease.

Sonneratia alba belong to the family Sonneratiacea. It is called mangrove apple or locally known as "*pagatpat*" or "*pedada*" in Malay. These species are found from East Africa throughout the Indian subcontinent, Southeast Asia, Northern Australia, Borneo and Pacific Islands [3]. Studies have shown promising mangrove species for the utilization as significant source of natural antioxidant [4][5]. But then there is no evidence in literature of studies made on *Sonneratia alba* leaf extracts for their antioxidant activity. This research gap and the abundance of *Sonneratia alba* make it an ideal plant to explore and consider for this medicinal application.

2. Materials and Methods

2.1 Preparation of Plant Extract

The fresh mature leaves of *Sonneratia alba* were collected from Kalawisan, Lapulapu City. The air dried leaves were placed in two glass jars, and were submerged separately in 95% ethyl alcohol and petroleum ether. These mixtures were allowed to stand for 48 hours while being agitated from time to time. Separately, the contents were filtered through a Buchner funnel and the extractives were concentrated to one-third its original volume using the rotary evaporator at 70°C at 60-70 rpm. After, they were placed in a vacufuge at 45^{o}C for solvent removal.

2.2 Preparation of DPPH Stock Solution

The initial DPPH concentration should be chosen to give absorbance values less than 1.0, which suggests a concentration of the stock solution in the range 50 *u*m to 100 *u*m [6]. In the preparation of 100*u*M DPPH-methanol stock solution, exactly 3.94 mg of DPPH powder was weighed and dissolved in sufficient amount of methanol to make a 100 mL solution.

2.3 Preparation of Test Solutions

Test solutions from the extracts having final concentrations of 300*u*g/mL were prepared for one

trial. About 1200*u*g of the ethanol-free extract was dissolved in sufficient amount of methanol to make two ml solution. Also 1200 *u*g of the petroleum ether-free extract was dissolved in sufficient amount of methanol to make two mL.

2.4 Preparation of Positive/Negative Control

To prepare ascorbic solution having final concentration of 300 *u*g/mL, about 1200 *ug* of ascorbic acid powder was dissolved in sufficient amount of methanol using a vacufuge tube to make two mL of a positive control good for one trial. Methanol was used as a negative control. The total amount of methanol in one trial was two mL.

2.5 DPPH Radical Scavenging Assay

The capacity of the cuvette is four mL. For optimum analytical accuracy, the final mixture was 2 ml of DPPH solution and 2 mL of the reductant [6]. Hence 2 mL of DPPH-methanol stock solution was prepared in four test tubes. Using a micropipette, 2 mL test solutions, positive control and negative control were added separately to each test tube. Three trials and three replicates were done for each of the test solutions and the controls.

After the addition of different solutions, the reaction was observed if free radicals have been scavenged by observing the color change from purple to yellow. Color reaction of each solution was observed and recorded. After tapping the tubes gently to facilitate the reaction, they were wrapped with aluminum foil. The tubes were allowed to stand for 20 minutes at room temperature. Each solution was transferred into the cuvette and the absorbance of the test solutions, positive control and negative control were immediately measured.

The reduction in the number of free radicals was measured by reading the absorbance at 515 nm using the UV-VIS spectrophotometer. Absorbance readings were recorded. A change of color from purple to yellow and a decrease in absorbance relative to the negative control were the two parameters used in the determination of the antioxidant activity of the test solution.

2.6 Determination of Percent Scavenging Activity

Percent scavenging activity is the extent of the ability of the sample to inhibit DPPH free radical oxidation reaction. The % scavenging activity of each test solution were calculated as follows [4][7][8]:

$$\text{Percent Scavenging Activity} = \frac{\text{absorbance of N} - \text{absorbance of TS}}{\text{absorbance of N}} \times 100 \quad (1)$$

(TS-test solution; N-negative control)

2.7 Phytochemical Screening

The ethanol-free extract which had positive antioxidant activity was qualitatively tested for the presence of alkaloids, anthraquinones, cardiac glycosides, flavonoids, saponins, steroids, tannins and terpenoids using the standard phytochemical methods from [9].

2.8 Partial Purification of the Ethanol-free Extract

For the column chromatography, silica gel was used as the stationary phase while chloroform-methanol (7:3) was the eluting solvent.

Every 5 mL of the fraction was collected. Thin-layer chromatography (TLC) using the same eluting solvent was performed on the collected fractions. Those fractions with the similar TLC profile based on the Rf values were pooled together for antioxidant assay – DPPH free radical scavenging assay. To identify the functional group present and possible compounds of the most bioactive fraction, FT-IR and GC-MS analysis were made.

2.9 Statistical Analysis

The data collected from the results were tabulated and expressed as means ± S.E. One-way Analysis of Variance (ANOVA) was performed. To further evaluate the differences of the groups, a post hoc analysis was done using Tukey HSD test. The level of confidence used was 99%.

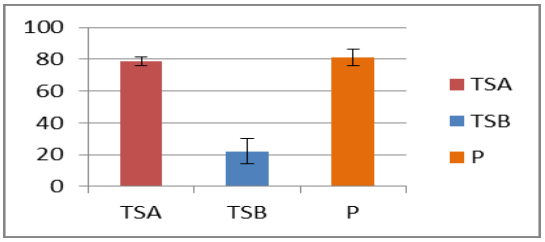
3. Results and Discussion

3.1 Crude Extracts in DPPH Assay

The ethanol-free extract (TSA) and positive control (P) showed a color change from purple to light yellow. Considering that the ethanol-free extract was able to reduce the stable radical DPPH to the yellow colored diphenylpicrylhydrazine then it does appear to possess hydrogen donating abilities that can act as antioxidants. The same crude extract had a lower mean value of absorbance reading (0.1054±0.014) closer to the positive control's (0.0932±0.026).

On the other hand, petroleum ether-free extract (TSB) and negative control (N) remained purple in color. The absorbance of the negative control showed the highest absorbance reading with mean value of 0.4983±0.039 followed by petroleum ether-free extract at 0.3856±0.024.

Graph 1. Mean Percent Scavenging Activity of Extracts



The graph shows TSA had a high mean percentage scavenging activity (78.76% ± 2.94) comparable to the P's (81.19% ± 5.29). The percentage scavenging activity is directly proportional to its antioxidant activity hence a high mean percentage activity would reflect good antioxidant activity.

3.1 Phytochemical Screening

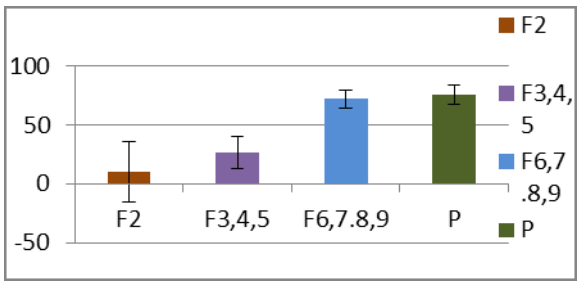
The phytochemical screening of ethanol-free extract which had the positive DPPH free radical scavenging activity revealed the presence of alkaloids, flavonoids, saponins, tannins and terpenoids. These were not exactly surprising results as [10] had mentioned that mangroves are a rich source of bioactive compounds such as triterpenes, saponins, flavonoids, alkaloids and tannins. Also using *S. alba* in their studies, [11][12] have validated the presence of terpenoids while [13] had confirmed tannin presence using the tannin colorimetric method on the fractions collected from the column chromatography of *Sonneratia. alba* methanol-free extracts.

3.2 Fractions in DPPH Assay

From the column chromatography, nine 5 mL fractions were obtained. Since fraction 1 didn't show any significant spot upon visualization under the UV light both in 254 and 365 nm, it was not included in the DPPH assay. Basing from the R_F values obtained from the fractions' TLC profiles, the following fractions (except for fraction 2) were combined together: fractions 3 to 5 and fractions 6 to 9. Three sets of fractions were subjected to DPPH free radical scavenging assay.

DPPH assay results revealed that only combined fractions 6, 7, 8 and 9 had similar results as the positive control (P) changing DPPH solution from purple to yellow color. The same fraction had a lower mean value of absorbance reading (0.10±0.02) than the negative control's (0.37±0.07). Also, this fraction showed the highest scavenging activity (72.06% ± 7.544) comparable to the positive control as shown in graph 2.

Graph 2. Mean Percent Scavenging Activity of Fractions



3.3 Statistical Analysis

The one way ANOVA analysis was used to compare the means of percent scavenging activity between the extracts and between the fractions in relation to the positive and negative control. A p-level of 6.50 x 10^{-27} ($p<0.01$) for the extracts and p-level of 1.23 x 10^{-18} ($p<0.01$) revealed that there were significant difference among the test solutions and fractions with respect to their antioxidant activities.

Using the Tukey HSD test, post hoc analysis determined which groups differ from each other. P-level of 0.3498 ($p>0.01$) for TSA vs positive control meant that there were insignificant differences among the means of their percent scavenging activity. TSA results were comparable with that of ascorbic acid solution. On the other hand, among the fractions, Tukey HSD test showed two groups (fractions 6, 7, 8 and 9 vs positive control and F2 vs negative control) having insignificant differences among their means. With a p-level of 0.5011 ($p>0.01$), fractions 6, 7, 8 and 9's results were comparable with the positive control's. As for fraction 2 vs negative control having p-level of 0.0216($p>0.01$), the mean percent of their scavenging activities have negligible differences.

3.2 FT-IR and GCMS Analysis

The FT-IR analyses of fractions 6, 7, 8 and 9 detected the absorption frequencies of its functional groups revealing the presence of possible compounds such as alcohol, aldehyde, alkane, alkene, aromatics, carboxylic acid, esters, ethers, ketones and phenols in the bioactive fraction.

Furthermore, GC-MS revealed thirty-two possible bioactive compounds basing on its peak area, molecular weight and molecular formula. From this GC-MS list, seven compounds were reported having antioxidant activities based on my literature research. They are namely: 1). Carvone ($C_{10}H_{16}O$); 2). Benzaldehyde, 2-hydroxy-4-methoxy-3, 6-dimethyl--($C_{10}H_{12}O_3$) ; 3). 2-Decenal, (E) - ($C_{10}H_{18}O$); 4). Falcarinol ($C_{17}H_{24}O$); 5). Azulene, 1, 4-dimethyl-7-(1-methylethyl)- ($C_{15}H_{18}$); 6). Phenol, 2, 6-bis (1,1-dimethylethyl)-4-ethyl-($C_{16}H_{26}O$); and 7). Oleic acid ($C_{18}H_{34}O_2$). Undoubtedly, the GC-MS and FT-IR results showed related findings with the functional groups present in the structures of the bioactive compounds with antioxidant activities.

Alkaloids, flavonoids, saponins, tannins and terpenoids were the secondary metabolites detected in the preliminary phytochemical screening of the crude ethanol-free extract. It is noteworthy to mention that these metabolites may be linked with the hydroxyl OH bond. Similarly, the functional group predominantly found in the bioactive compounds with antioxidant activity is also the hydroxyl group for phenols. Phenolic compounds are proton free radicals. Plant polyphenols have drawn increasing attention due to their potent antioxidant properties and their marked effects in the prevention of various oxidative stress associated diseases [14][15][16][17][18].Therefore, these results suggest that phenolic compounds may be

associated with antioxidant activity of the ethanol-free extract of *Sonneratia alba* leaves.

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Characteristics of Electrochemical Double Layer Capacitor Using Multiwalled Carbon Nanotubes

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Abstract **–** An electrochemical double layer capacitor (EDLC) cell made from an electrode of 90 wt.% of the multiwalled commercial carbon nanotubes, 10 wt.% of poly (vinylidene fluoride-co-hexafluoropropylene) and polymer electrolyte made up of a compositions of 60 wt.% of PVA/H_3PO_4 and filter paper was characterized in this study. The electrical conductivity of solid polymer sample delivered an outstanding conductivity of 1.47×10^{-4} Scm^{-1}. Cyclic voltammetry (CV) measurement of cell shows a high specific capacitance of 23 Fg^{-1} for the scan rates 10 mV was recorded from the cell. However, when the galvanostatic charge/discharge (GCD) was conducted on the cell, it's specific capacitance reduced to 18.0 Fg^{-1} at the discharge current of 10 mA with a specific energy and power densities of 5.25 Whg^{-1} and 0.08 Wg^{-1} respectively, at a discharge current of 100 mA. Its overall columbic efficiency of 88.9 % is recorded. Finally the cell delivered the best cycling stability with about 85 % capacitance retention of its initial value after 5000 cycles which could be as result of the high conductivity of the polymer electrolyte.

1. Introduction

With the global dramatic exponential increase in the development of portable electronics, such as e-paper and other flexible devices, power sources with superior flexibility become an important recent demand [1]. Due to their higher power density [2], larger capacitance, longer cycleability, shorter charging/discharging time and zero harm to the environment [3], supercapacitors have been the research focus in the area of international clean energy and attracted more and more attention from both academia and industry all over the world in recent years. Among the electrode materials used in supercapacitor fabrication are carbon–based materials such as activated carbon and carbon nanotubes (CNTs). Although activated carbon with high surface area exhibits high specific capacitance at a very low discharge current, however poor rate of capability hinders its further application. On the other hand, CNTs possess a very good electrical conductivity and straight large pore channels (mostly mesopore size), which are favorable for ion diffusion and charge propagation, enabling them outstanding rate performances [4].

The amount of electrical charge accumulated due to electrostatic attraction in EDLC depends on the area of the electrode/electrolyte interface that can be accessed by the charge carriers. The higher surface area of the electrode material could leads to higher capacitance if the area can be fully accessed by the charge carriers. However, higher surface area does not always result in higher capacitance, because the capacitance depends on the pore size and its size distribution. The surface area is hardly accessible if it consists of micropores (<2 nm). This is what made CNT-based electrodes as good materials for EDLC cell as mentioned above. Functionalization is one of the methods used to further enhance the capacitance of CNT-based EDLC. The value of specific capacitance may increase significantly after strong oxidation in nitride acid for example, due to the increase of the functional groups on the CNT's surface [5].

The purpose of this work is to investigate the characteristics of the EDLC cell that is made from commercial and a functionalized multiwalled CNTs with solid polymer electrolyte of aqueous formation. Aqueous electrolytes were selected in this work due to their high ionic conductivity which could result to the high specific power of an EDLC. Although several works on the EDLC fabrication based on CNTs, there are few occasions where functionalized-based electrodes are reported.

2. Experimental

2.1 Preparation

2.1.1 Electrolytic preparation

The preparation of the polymer electrolyte was highlighted else were [6-7]. However for more elaboration, the electrolytes were prepared as follows; the active materials comprise of H_3PO_4 and PVA. The H_3PO_4 functions as an ionic liquid, while PVA acts as a polymeric matrix, allowing ionic transport while simultaneously functioning as a separator between the electrodes of the supercapacitor. H_3PO_4 (>85 wt.% in water, molar mass of 98.00 g/mole, product, number of 1502-80) was obtained in aqueous form from R & M marketing, Essex, UK brand, while the PVA

(molecular weight; 89,000-98,000, 99+ % hydrolyzed) was obtained from Sigma Aldrich. The advantage of the aqueous electrolyte is its higher conductance (0.8 S cm^{-1} for H_2SO_4) and the fact that purification and drying processes during production are quite easier compare to other types of electrolytes. Again, aqueous electrolytes are known to be cost effective than organic electrolyte. Both H_3PO_4 and PVA were used as received without further treatment or purification.

An aqueous solution of PVA is prepared by combining PVA with distilled water in the ratio of 1:10 by volume. This solution is mechanically agitated by magnetic stirring at 60 °C for five hours to thoroughly dissolve the PVA in the distilled water. H_3PO_4 was then mixed with the PVA aqueous solution in the ratio of 60:40 wt%. The mixing is done in a drop-wise manner with magnetic stirring at 60 °C for about one hour or thereabouts until it completely turns to homogenous solution.

The mixture is then allow to cool down to an ambient temperature. The resulting homogenous solution of PVA/H3PO4 is cast over a plastic Petri dish. Prior to this, the Petri dish was scraped off the dust using a tissue soaked in acetone. The PVA/H3PO4 solution solidifies onto the Petri dish and upon curing for about 4 weeks at room temperature. Prior to that also, a cellulose filter paper (Whatman brand) is cut into a 6 cm x 5.5 cm and soaked in a segment of the aforementioned solution. The solid layer was easily peeled off from the Petri dish after it dries as a freestanding layer. The thickness of the solid layers formed in this procedure was controlled to about 0.019 ± 0.002 mm. The resulting polymer film was then put in the plastic bag for safe keeping and to avoid contamination from the surrounding, and for further property analysis.

2.1.2 Sample preparation of the Electrode Material

The commercial functionalized multiwalled CNTs with -COOH content of 0.49 wt.%, an outer diameter of > 50 nm, length of 10-20 μm, purity and Ash are both >95 wt.%, and <1.5 wt.%, respectively, Surface area of >40 m^2g^{-1} and Conductivity of >10^2 Scm^{-1} was purchased locally in Malaysia (with material code MC8/21/20). The binder used, was poly(vinylidene fluoride-co-hexafluoropropylene) P(VdF-HFP) (with average molecular weight of ~400,000; Mn of ~130,000 pellets; product number of 427160) was purchased from Sigma Aldrich. So, the double layer capacitor was made with a mixture of 90 wt. % of the functionalized MWCNTs and 10 wt. % of P(VdF-HFP), mixed inside a 20 ml of the acetone. The slurry was then cast onto to the Aluminum foil and allowed to dry for about two hours at room temperature. Prior to that, an applicator was used to polish the slurry with the view to leveling it and obtaining a desired thickness which was around 0.127 ± 0.002 mm. The dried sample was then further dried in an oven for about 12 hours at a temperature of 100 ^{0}C. Afterward, the solid films were obtained and were further cut into 2 cm^2 x 3 cm^2 each. The weights of the films were measured by means of a micro-balance (Santorius, Ax 224) with an accuracy of 0.001 mg. The average weights of two electrode films that make a cell was around 0.224 g. Using a Perspex of about 5 cm x 4 cm, the cell was set up by sandwiching two electrodes with the electrolyte and assembled in an innovative supercapacitor tester.

2.1 Characterization

Conductivity measurement was carried out on solid polymer electrolyte film which is composed 60 percentage ratio of PVA-H_3PO_4, an impedance method which also was highlighted by [8]. The solid polymer film was sandwiched between a stainless steel, ion-blocking liked measuring device (otherwise known as a probe), each of surface area 2 cm^2 in radius. The impedance measurement was carried out by using Electrochemical Impedance Spectroscopy (EIS) testing machine named HIOKI 3532-50 LCR Hi-Tester which was connected to the computer. The frequency range of the software was 50 Hz - 1 MHz, and simultaneously calculating both real and imaginary impedance. The electrical conductivity of solid polymer sample was calculated using the following equation;

$$i = \frac{t}{R_b A} \tag{1}$$

were i is the conductivity of the sample, t, R_b and A are the thickness, the bulk resistance and area of the samples respectively. The thickness of the sample was measured twice at different positions of the polymer films and an average was taken using digital micrometer.

Furthermore, the electrochemical behavior of the cell for the CV analysis and CD were respectively carried out using a newer battery charger which has been interfaced to a computer called “e-machines” (model: ET1850, Rating: 100-127/220-240 Vac, 6/3.15 A (6/3, 15 A), 60/50 Hz) and Gamry instrument Framework. The specific capacitance of the electrode materials obtained in the two-electrode system was calculated by integrating the CV curves using the equation;

$$C_{sp} = \frac{2i}{sm} \tag{2}$$

where i is the current difference, m is the mass of the electrode and s is the scan rate. However, the effective capacitance obtained from each CD profile is is calculated using the following equation;

$$C_e = i\frac{\Delta t}{\Delta V} \quad (3)$$

where C_e stands for effective capacitance, i is the applied current and Δt and ΔV are the changes in time and current respectively. While power and energy densities were obtained using the relations earlier stated in the previous chapter. Moreso, the columbic efficiency is calculated using the following equation;

$$\eta = \frac{t_d}{t_c} \times 100 \quad (4)$$

where t_d and t_c represent the time of charging and discharging respectively.

3. Results and Discussion

3.1 Conductivity Analysis of the Solid Polymer Electrolyte

Fig. 3.1 depicts an impedance plots (Nyquist plot) of a sample polymer electrolyte which has cellulose incorporated in it. This figure consisted of 60 wt.% of the H_3PO_4 at room temperature. It can be seen from this figure that there affair no any semicircle and the calculated bulk resistance was very low (43.6 Ω), thereby resulting in an outstanding electrical conductivity (1.47 x 10^{-4} Scm^{-1}). This results could be as result of the optimization of the acid concentration in the sample, which is an evident that, this electrolyte can be used as separator in the EDLC cell assembly.

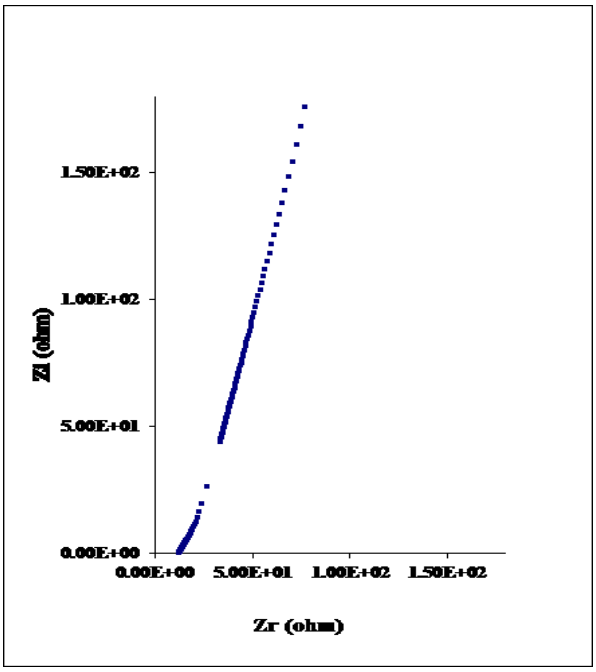

Figure 3.1: Impedance plots of solid polymer electrolyte containing 60 wt.% ratio of the H_3PO_4 at room temperatures.

3.1 Cycle Voltammetry of the cell

Fig. 3.2 also shows CV of cell at the scan rates of 10, 50 and 100 mV for a voltage window of 0.0 – 1.0 V. High specific capacitances of 16, 19 and 23 Fg^{-1} for the scan rates of 100, 50 and 10 mV respectively was recorded from the cell (see also Table 3.1). From these results, it can be deduce that, there is relationship between the capacitance and scan rate. The specific capacitance decreased from 23 to 16 Fg^{-1} which could be attributed to the slow transfer of the ions on the electrode/electrolyte interface. The resulting CV curves of this cell displayed a better mirror symmetric indicating an ideal supercapacitive behavior for a commercial functionalized CNTs that uses an aqueous electrolyte of 60 wt.% of H_3PO_4 in homogenous solution of PVA/H_3PO_4.

Table 3.1: CV Performances of the cell

Cell	Working voltage (V)	Capacitance value of different scan rates (Fg^{-1})		
		100 mV	50 mV	10 mV
1	1	16	19	23

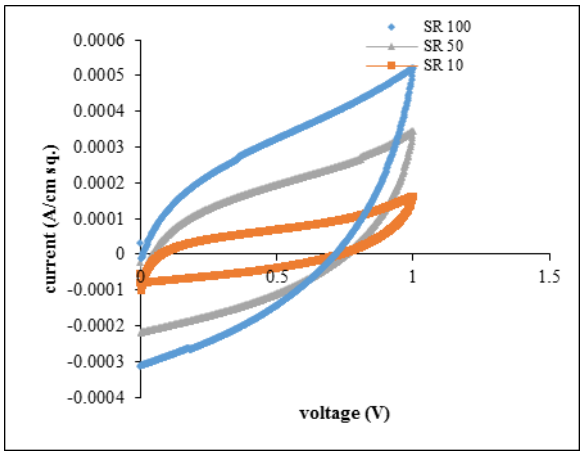

Figure 3.2: CV of the EDLC at the scan rates of 10, 50 and 100 mV.

3.2 Galvanostatic Charge/Discharge of the cell

GCD is the accepted measurement method for determining capacitance for packaged supercapacitor in the supercapacitor industry and correlates more closely to how a load is typically applied to such supercapacitor in the majority of applications. Most importantly the same voltage range should be used for testing should match that used for commercial cells and should reflect the electrolyte's electrochemical window. For instance, an electrochemical window from 0.0 V to approximately 1.0 V, should be used for aqueous electrolytes and 0.0 V to 2.5–2.7 V for organic electrolytes. This is because, driving a cell above its true maximum operating voltage can lead to

an overestimation of specific capacitance and cells operated at these levels will have shortened lifetimes and poor efficiencies due to the non-reversible reactions within the cell. Significant errors can also be introduced by the method used to calculate the slope (DV/dt) [9].

Fig. 3.3 shows a CD profile of the cell. Although, the variation of voltage with respect to time is not linear due to the porous nature of the electrode materials which is in conformity with the argument put by Pan et al. (2010), the profile, however, shows good perfection. This could be as a result of improvement in the conductivity of the electrolyte. Moreso, the reduction of ERS can be attributed to the improvement in conductivity because of the carrier induced by the functionalization [5]. It's charging and discharging times are observed to be the same, and the specific capacitance of 2.4, 6.0 and 18.0 Fg^{-1} are obtained at discharge current of 100, 20 and 10 mA respectively, with a specific energy and power densities of 5.25 Whg^{-1} and 0.08 Wg^{-1} respectively, at a discharge current of 100 mA. Its overall columbic efficiency of 88.9 % is recorded. However, the voltage drop noticed at the beginning of the discharge curve could be as a result of improvement in the conductivity of the electrolyte.

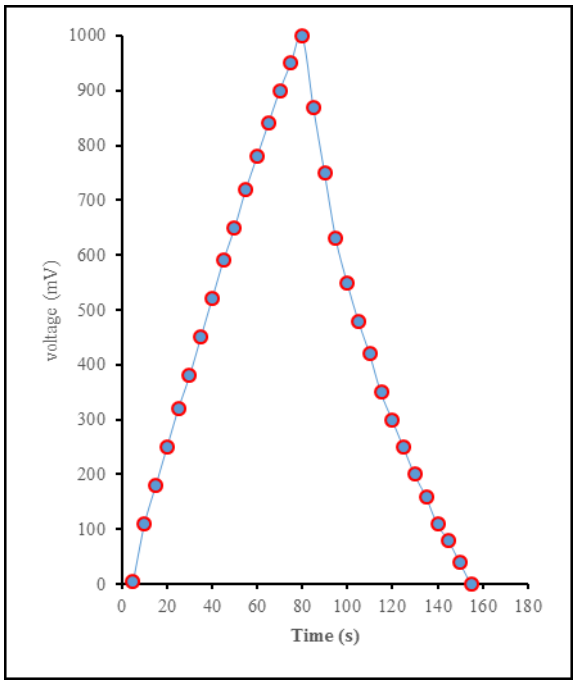

Figure 3.3: CD graph of the cell at the discharged current of 100 mA.

Fig. 3.3 shows a CD profile of the cell. Although, the variation of voltage with respect to time is not linear due to the porous nature of the electrode materials which is in conformity with the argument put by Pan et al. (2010), the profile, however, shows good perfection. This could be as a result of improvement in the conductivity of the electrolyte. Moreso, the reduction of ERS can be attributed to the improvement in conductivity because of the carrier induced by the functionalization [5]. It's charging and discharging times are observed to be the same, and the specific capacitance of 2.4, 6.0 and 18.0 Fg^{-1} are obtained at discharge current of 100, 20 and 10 mA respectively, with a specific energy and power densities of 5.25 Whg^{-1} and 0.08 Wg^{-1} respectively, at a discharge current of 100 mA. Its overall columbic efficiency of 88.9 % is recorded. However, the voltage drop noticed at the beginning of the discharge curve could be as a result of improvement in the conductivity of the electrolyte.

One of the criteria in supercapacitor's fabrication and application is its ability to endure long term cycling stability. In this respect, the cycling endurance measurement over 5000 cycles for the cell A (as shown in Fig. 3.4) was conducted using the CD test at the working voltage of 1 V. This graph showcased the best cycling stability with about 85 % capacitance retention of its initial value after 5000 cycles which could be as result of the high conductivity of the polymer electrolyte.

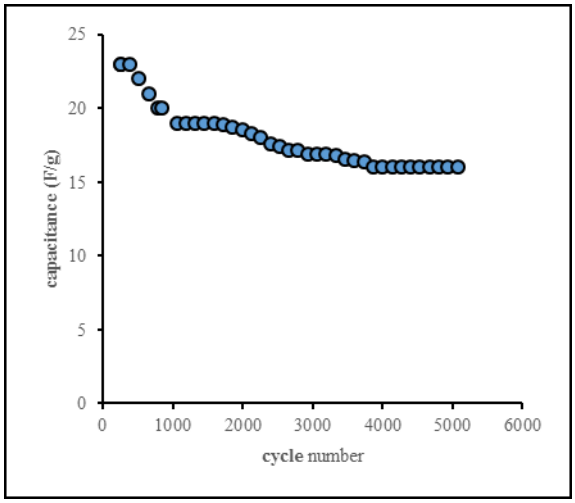

Figure 3.4: Cyclic performances of cell for a working voltage of 1 V.

4. Summary

In this study, we have demonstrated the characterization of EDLC cell made from a commercial MWCNTs and solid polymer electrolyte. The electrical conductivity of solid polymer sample measured shows that, the electrolyte has an outstanding conductivity of 1.47 x 10^{-4} Scm^{-1} which could be as a results could be as result of the optimization of the acid concentration in the sample, which is an evident that, this electrolyte can be used as separator in the EDLC cell assembly. CV measurement of cell shows a high specific capacitances of 16, 19 and 23 Fg^{-1} for the scan rates of 100, 50 and 10 mV

respectively was recorded from the cell. However, the when the GCD was conducted on the cell, it's the specific capacitance reduced to 2.4, 6.0 and 18.0 Fg^{-1} at the discharge current of 100, 20 and 10 mA respectively, with a specific energy and power densities of 5.25 Whg^{-1} and 0.08 Wg^{-1} respectively, at a discharge current of 100 mA. Its overall columbic efficiency of 88.9 % is recorded. Finally the cell delivered the best cycling stability with about 85 % capacitance retention of its initial value after 5000 cycles which could be as result of the high conductivity of the polymer electrolyte.

5. References

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Enzymatic Hydrolysis of *Jatropha curcas* Seed Cake and Utilisation of Its Hydrolysates for Single Cell Protein Production

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Abstract - Production of jatropha seed cake hydrolyzates (JSCH) and its utilisation for a medium in single cell protein (SCP) production was examined. To produce JSCH, 5% of jatropha seed cake (JSC) powder was enzymatically hydrolysed using concentrated crude enzyme from *Trichoderma viride*. Hydrolysis released 27.3 mg/ml reducing sugar when done at 30^{o}C for 36 hours. JSCH consisted of 16.9 g/ml or 62% sugar as monosaccharide from the total reducing sugar produced, as analysed using Gas Chromatograph. Based on these results, the sugar-rich JSCH was then used for medium SCP *Saccharomyces cerevisiae* production. Aerobically fermentation at 30^{o}C and 120 rpm shaking for 48 hours produced 12.5 mg/ml SCP with efficiency 74.2%, respectively.

Keywords: Jatropha, Hydrolysate, SCP, fermentation

1. Introduction

In *J. curcas* oil processing, a significant quantity organic stuffs remain as polysaccharide and protein rich-JSC [1] is disposed as waste. JSC has been reported to possess phorbol esters [2], a potenial toxic compound to animals so that cannot be used as feed because of its toxic properties [3], [4]. These toxicities of JSC has been studied extensively in animals. When animals (e.g; fish, sheep and goat) were fed on phorbol ester containing feed, it could make decreasing of glucose level, dehydration diarrhea, and other anti-tick feeding effects [3], [5]. However, JSC is a source which can be used as organic fertilizer due to its high nitrogen content [6] [7], [8]. Therefore, it should be reasonably source for other useful products.

Due to the environmental concern, effective biological methods to manage and solve some of the environmental problems and other forms of pollution should be considered. Through bioconversion, the waste material can be converted to other useful product [9], which may also reduce the processing cost. Another important advantage of bioconversion that used some organisms such as fungi, bacteria or yeast is a long history of safety aspect of usage for the manufacture of food products destined for human consumption and is regarded to be nontoxic and nonpathogenic.

In the present study, utilizing JSC for producing ″sugar rich JSCH″ by hydrolysis using crude enzyme from *T. viride* and converting to SCP was reported.

2. Materials and Methods

2.1 Enzyme Production

Jatropha seed cake medium and *T. viride* were used for producing crude enzyme. In this step, optimisation of cultivation and harvesting of crude enzyme were done in series of days to obtain the crude enzyme with optimum activity. The activity was measured by reducing sugar produced during JSC hydrolysis. Detail of this step will be explained in Results and Discussion.

2.2 Degree of Hydrolysis and Total Sugar Analysis

The degree of hydrolysis was examined by incubating the reaction mixture of concentrated crude enzyme and JSC substrate at 30^{o}C. The JSCH as reducing sugars measured by the method of Nelson [10] as modified by Somogyi [11] using glucose as a standard sugar. The degree of hydrolysis of JSC was calculated as follows.

$$DH(\%) = \frac{TRS\,(w/v)}{TS\,(w/v)} * 100\% \qquad (1)$$

DH = Degree of Hydrolysis
TRS = Total Reducing Sugar
TS = Total Substrate

The total sugar content of JSCH was also measured according the phenol-sulphuric acid method [12].

2.3 Analysis of Monosaccharides

Analysis of sugar as monosaccharides was performed by using Gas Chromatograph as alditol acetates [13], [14] with a few modifications [15].

2.4 JSCH Production Medium for SCP Production

Five percent of JSC was hydrolyzed with concentrated crude enzyme at 30^{o}C under unbuffered condition for 48 hours and reducing sugar production was monitored every 6 hours. The total sugar also quantified using GC as described above.

2.5 Maintaining SCP

Saccharomycess cerevisiae was maintained at 30°C on 1.5% agar medium pH 6, with yeast-extract (0.3%), malt extract (0.3%), pepton (0.5%) and glucose (1%).

2.6 Analysis of SCP Production

Saccharomycess cerevisiae was cultured on JSCH medium aerobically in 500-ml shake flask using shaker set at 120 rpm and 30°C. Growth or biomass (mg/l) of *S. cerevisiae* was observed by measuring absorbance at 660 nm for every 12 hours. To relate the measured absorbance to biomass, the method of Kim *et al*. 1998 was adopted [16]. The sugar assimilation ability of *S. cerevisiae* SCP was determined by measuring the remaining of reducing sugar and GC analysis.

The assimilated sugar and assimilation efficiency were calculated as follows.

$$AS(g) = IS(g) - RS(g) \qquad (2)$$

$$AE(\%) = \left(\frac{AS(g)}{TS(g)} \right) * 100\% \qquad (3)$$

And the maximum yield of biomass was calculated as formula below.

$$Max.\ yield\ of\ biomass(\%) = \frac{Max.\ biomass(g)}{AS(g)} * 100\% \quad (4)$$

AS : Assimilated Sugar
IS : Initial Sugar
AE : Assimilation Efficiency
TS : Total Sugar

3. Results and Discussion

Enzymatically hydrolysis of JSC has been evaluated on this research. *T. viride* was seleted on this research because the genus *Trichoderma* easily grow and exploit the carbon as well as nitrogen from various biomass [17], [18]. Also, this species well known produced board spectrum of extracellular enzyme such as cellulase [18], [19], [20], [21], [22], [23], [24], xylanase [25], [26], exoglucanases and endoglucanases [27] and some hemicelluloses.

In this research, optimum crude enzyme production and activity of *T. viride* based on the measurement reducing sugar released during hydrolysis against JSC substrate. To optimise crude enzyme production, the solid state fementation was done on 10g JSC on flask and inoculated with *T. viride,* then incubated at 30°C. The enzyme was harvested and the activity daily monitored.

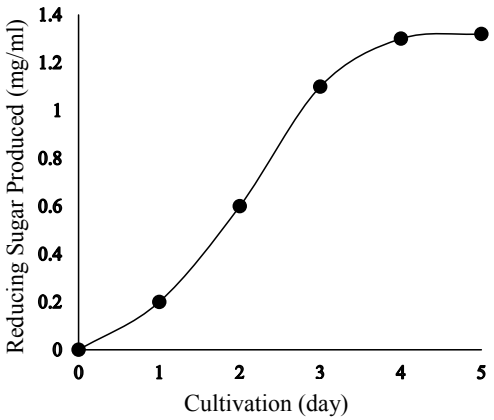

Figure 1. Optimisation of Enzyme Production of *T. viride*

It was observed and shown at Figure 1 that optimum extracellular production was found in fourth day cultivation. The enzyme able to hydrolyse JSC and produced reducing sugar at 1.3 mg/ml when hydrolysis of 1% JSC substrate was done in acetate buffer 50mM pH 5, incubated 37°C for 30 minutes.

According to optimisation result, large scale of extracellular enzyme production was done on 500 g JSC inoculated by *T. viride*, incubated at the same condition on 30°C for 4 days. The extracellular crude enzyme was harvested by 1% NaCl 500 ml and 0.1% toluene (v/v), followed by shaking at room temperature for 12 hours. The suspension was fitered and centrifuged 4000 rpm for 10 minutes to recover the supernatant as a source crude enzyme. Then the crude enzyme was concentrated to about one-tenth of the initial volume by ammonium sulphate precipitation at 70% saturation. The precipitate was dissolved and dialyzed against distilled water for 3 days. This solution was stored at 4°C till used for JSC hydrolysis.

Hydrolysis of 5% JSC was done without adjustment of pH resulting in 27.3 mg/ml reducing sugar in JSCH when done at 30°C for 36 hours. This evidence proved that concentrated enzyme hydrolysed JSC readily with the degree of hydrolysis 78%. Further analysis showed that 62% or 16.9 g/ml of JSCH is monosaccharide as analysed using GC. It was also elucidated by thin layer chromatography (TLC) that the spot was detected as glucose in JSCH (data not shown).

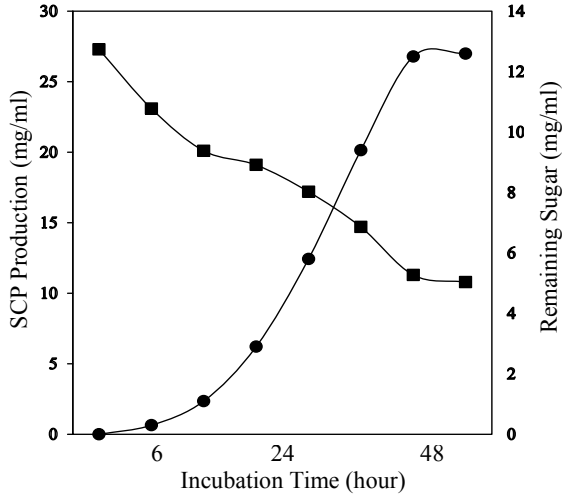

Figure 2. Growth Pattern of *S. cerevisiae* (●) and Remaining Sugar (■)

Tabel 1. SCP Production and Remaining Sugars

Incubation (Hour)	*SCP (mg/ml)*	*Sugars (mg/ml)*	
		Reducing Sugar	*Monosaccharides*
0	0	27.3	16.9
6	0.3	23.1	13.1
12	1.1	20.1	10.4
18	2.9	19.1	7.2
24	5.8	17.2	3.2
36	9.4	14.7	2.1
48	12.5	11.3	1.9
60	12.6	10.8	1.2

For application purposes, the SCP production was done with sugar-rich JSCH medium without any nutrient added. Growth of SCP *S. cerevisiae* biomass in medium was monitored every 6 hours. And remaining sugar was also quantified to ensure assimilation by *S. cerevisiae* happened which indicated decreasing amount of sugar in JSCH. This fermentation was done at 30^{o}C and 120 rpm shaking aerobically. Observation showed that SCP was produced maximum at 48 hours. As estimated at OD 660nm, the growth or biomass maximum of *S. cerevisiae* was 12.5 mg/ml. It were reported that some *yeast* [28], [29], [30]. Figure 2 showed that the remaining sugar also decrease gradually as reflected sugar utilisation occurred for SCP growth. As shown in Table 1, monosaccharides also decrease accordingly and 1.2 mg/ml remained after 60 hours. This indication revealed that *S. cerevisiae* assimilated monosaccharides in JSCH definitely. It was calculated that SCP production gave efficiency 74.2%, respectively.

4. Conclusion

Microbial utilisation of agricultural wastes JSC biomass to protein-rich food has been shown in this paper. The result of 62% sugar as monosaccharide production through enzymatic hydrolysis of 5% JSC and conversion of its hydrolysate to SCP production resulting in efficiency 74.2% which praiseworthy enough. However, scaling up to be industrially as well as optimizing and including the process with the point of view to increase in economic value must be evaluated.

5. Acknowledgments

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Variation of Dopant Anions on Polypyrrole-Based Humidity Sensor: Polymerisation and Characterisation

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Abstract **-** Humidity measurement is very important for human daily life and other industrial applications. Investigations of new material for humidity sensor have to be continuously proceeded to improve the sensor's performance and to meet the demand of the humidity sensor in the market. Current progress in development of humidity sensor based on doped polypyrrole is reported in this paper. Three different anions, fluoroborate, toluene sulphonate and perchlorate, have been used as dopant by dissolving the appropriate organic salt in pyrrole solution prior electropolymerisation process. Cyclic voltammetric technique was used to enable polarization of the electrode in an electrochemical cell at certain potential range and sweep rate. Gold-coated cooper electrode was used as working electrode for depositing the resulted polypyrrole. Resistance measurement of the doped polypyrrole resulted was carried out by measuring its DC resistance of the polypyrrole which was deposited as a bridge between two cooper conductors. Chemical properties and concentration of dopants are important parameters affecting physical properties of the polymer and succesfulness of the polymerisation process. Generally it was noted that resistance of the doped-popypyrrole decreased as dopant concentration increased. The three doped polypyrroles have shown linear response to humidity variation in a range 30%-90% RH, however it was indicated that repeatability of perchlorate-doped polypyrrole was the best among the others. Methanol was found as the highest threat of interfering volatile chemical affecting to the polymer response to humidity.

Keywords **:** humidity sensor, polypyrrole, electropolymerisation, voltammetric.

1. Background

Water vapour in air environment is an important factor for the well-being of humans, animals and others life organisms. Level of comfort related to water content in air environment is determined by a combination of two factors namley humidity and ambient temperature. Humidity is also an important factor for operating certain equipment, for instance, high impedance electronic circuits, electrostatic sensitive components, high voltage device, etc. Hence, measurement and control of humidity are important not only for human comfort but also for a broad spectrum of industries and technologies[1].

It was also reported that during the 1990s both supply of and demand for humidity sensors have shown a large increase, the rise has been due to an increase in volume in traditional areas as well as to the emergence of numerous new applications[2]. The use of humidity control system has greatly increased in the quality control of production processes in a wide range of industries and intelligent control of the living environment in building, where humidity sensors are used to maintain a comfortable humidity level and cooling[3]. It was considered that the best type of the humidity sensors were based on the electrical properties such as resistance and capacitance and those are best suited to modern automatic control systems [4].

According to the above facts and reasons, development of humidity sensors is also still necessary to be carried out due to some drawbacks of the current sensors that should be minimised which is mainly affected by their sensing element material as reported by some researchers[2,4]. Hence, investigations of new material sensor or combination of some materials have to be continuously proceeded to improve the sensor performance characteristics.

Conducting polymers materials such as polypyrrole, has attracted some researchers exploring this material for sensor with various target analytes[5,6,7]. It was also reported that some of conducting polymers have characteristics that can be further exploited as humidity sensor[8,9,10]. This paper deals with a current on going progress of development of humidity sensor based on a particular conducting polymer i.e. polypyrrole that employing some type of dopant materials.

2. Materials and Methods

Materials and reagents

Monomer pyrrole (Merck) was used without purification to produce polypyrrole by adding some dopant materials: perchlorate, para-toluene sulphonate

and tetrafluoroborate. All chemicals were dissolved in acetononitrile.

Electropolymerisation technique of polymer

Electropolymerisation of polypyrrole was conducted using potentiodynamic cyclic voltammetry technique performed on a computer-controlled potensiostat Amel 433A®. Electrochemical cell that consists of gold-coated cooper working electrode (WE), stainless steel counter electrode (CE) and Ag/AgCl reference electrode (RE), immersed in pyrrole solution and the appropriate dopant at varied concentration.

Characterisation of polypyrrole-based sensor

Characterisations of polypyrrole-based sensor to humidity variation were conducted by placing the sensor in a humidity-controlled closed container and measuring resistance change of the sensor every varied value of the humidity. Continuous recording of the resistance sensor response during characterisation was performed using computer-connected digital multimeter (BM 202 Brymen®) that enable storing bulk resistance data into the computer storage for further data processing. Controlling a certain value of humidity in the test chamber was achieved by using saturated salt solution method[11].

4. Results and Discussion

Successfulness of depositing the polymer onto electrode surface was known by monitoring the voltammogram of polymerization process and physical change of the electrode surface. A selected series of voltammogram of three dopant used at concentration of 0.2M was shown in Figure 1.

As shown in figure 1 below a good regular shape voltammogram was obtained from the pyrrole solution containing 0.2M of each dopant. It is an indication that electropolymerisation has proceeded as expected, and the polymer produced would be in a good quality. A similar condition was also resulted from the solution with dopant concentration at 0.1M and 0.05M of ClO_4, 0.1M of BF_4(not shown). According to physical investigation the polymer formation during potential cyclic, it was noted that the polymer was deposited at first cyclic covering the electrode as well as the electrode gap although it was still black transparent. The polymer colour was getting darker as the number of cyclic increased. Concentration of these two dopants also affected the thickness as well as the colour of the deposited polymer. A thicker and darker polymer was obtained from more concentrated dopant. A slightly different result was obtained from dopant P-TS, in which its voltammogram as well as the deposited polymer were not as good as the polymer produced from dopant BF_4 and ClO_4. It was also observed that the quality of its voltammogram was decreased as P-TS concentration lowered. All of polymers produced using dopant P-TS did not fully covering the tiny gap of the electrode hence conductivity between the separated electrode was not fully facilitated. A similar result was observed when dopant BF_4 lowered to 0.05M.

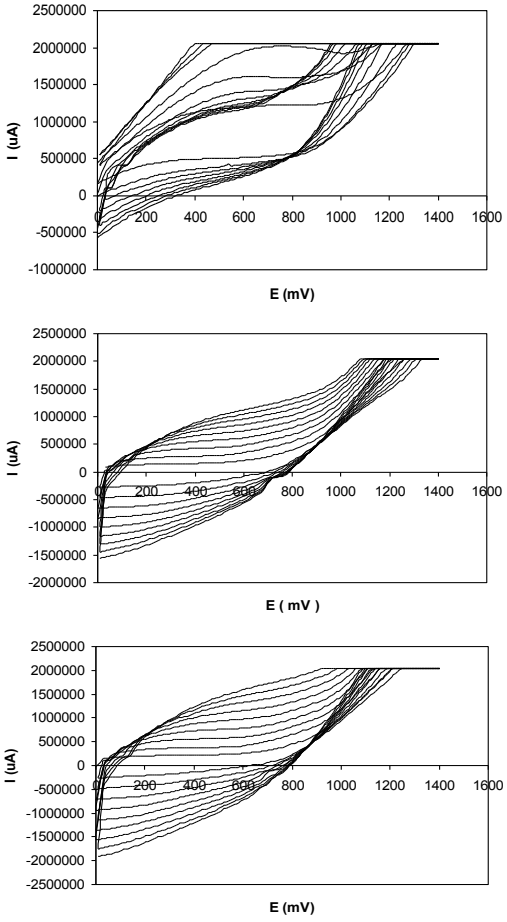

Figure 1. Selected voltammogram of polymerisa-tion process of polypyrrol doped with 0.2M P-TS (top), ClO_4 (middle), and BF_4 (bottom).

Chemical properties and concentration of dopant are important parameters affecting physical properties of the polymer. It was also observed that the dopant concentration contributing to the current flowing as indicated by the voltammogram. Dopant concentration is a main factor affecting electrolyte property of pyrrole solution. In other word ion concentration of the solution increase as dopant concentration increase. As a consequence polymerisation process will be enhanced. This phenomenon is in good agreement with previous report [12].

Resistance of the doped-popypyrrole decreased as dopant concentration increased as shown in Table 1. Decreasing resistance or increasing conductance of the doped-poypyrrole is probably caused by increasing amount of anion of the dopant constructing chemical bonding with polymer chain. Furhermore this condition enhancing interaction between positive charge of the polymer and negative charge of anion dopant, hence lowering the polymer resistance.

Table 1. Resistance change of doped-polypyrrole due to variation of humidity and dopant concentration of 0.2M(I), 0.1M (II), and 0.05M(III) appropriate dopant.

RH (%)	Resistance (Ohm)								
	ClO_4^-			p-Ts^-			BF_4^-		
	I	II	III	I	II	III	I	II	III
30,5	14	114	173	14	24	429	18	21	2185
52,0	18	118	184	15	25	432	21	23	2240
71,2	26	122	189	20	28	441	26	28	2346
90,0	37	124	203	22	31	451	29	30	2380

According to data obtained as shown in Table 1, it is clear that a similar trend of resistance change of the doped-polypyrrole has been observed. All types and concentrations of dopant used for doping polypyrrole have indicated a resistance increase when humidity was increased. A series of selected characterisation results when the polypyrrole exposed to various value of relative humidity was shown in Figure 2.

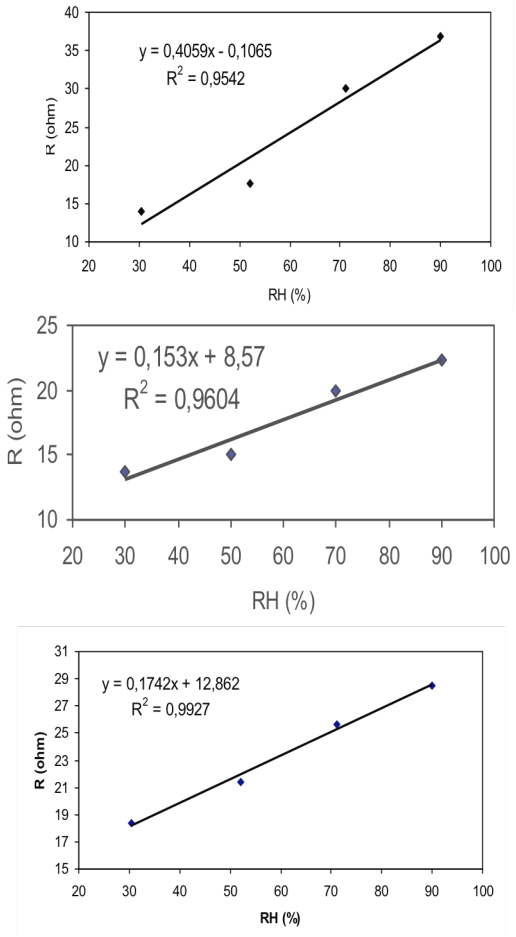

Figure 2. Characteristic response of doped-polypyrrole to RH variation, at dopant concentration of 0.02M: ClO4 (top), P-TS (middle), and BF4 (bottom).

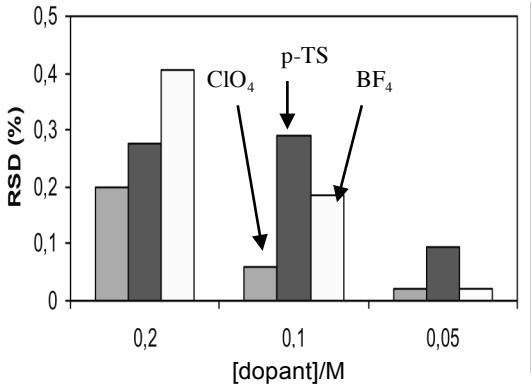

Figure 3. RSD of PPy/ClO_4, PPy/p-TS dan PPy/BF_4 calculated based on 6 measurements.

Generally PPy/ClO_4, PPy/p-Ts and PPy/BF4 have shown good repeatability as indicated by their relative standart deviation (RSD) based on 6 measurements (Figure 3).

Repeatability of PPy/ClO4 was the best among PPy/p-Ts and PPy/BF4. This evidence perhaps correlated to the polymerisation process in which PPy/ClO4 was the best as indicated by its voltammogram.

Investigation of some interference volatile chemicals was carried out by injecting the interference into a controlled-humidity chamber set at 72.1%. Resistance change due to the presence of interference was then recorded. Some volatile chemical were used namely toluene, chloroform, methanol, ethanol dan 2-propanol. For this purpose, only PPy/ClO_4 0,2 M was investigated. Results that shown at Table 2 has indicated that all volatile chemicals used contributing to increase resistance of PPy/ClO_4 at different magnitude. A biggest resistance change was resulted from methanol whereas the smallest contribution coming from 2-propanol.

Table 2. Effect of some gas interferences to resistance change of polypyrrole

n	Resistance								
	Ω0	Ω1	Ω0	Ω2	Ω0	Ω3	Ω0	Ω4	Ω0
1	104,3	105,3	105,0	109,0	103,0	113,0	99,6	100,2	114,5
2	104,8	104,8	105,0	108,5	103,2	113,6	99,7	100,2	114,7

Ω_0 = exposed to RH 72,1 %; Ω_x = exposed to gas interferences: 1=toluene; 2=chloroform; 3=methanol; 4=ethanol; 5=2-propanol

5. Conclusion

Dopant is very important material for controlling polymerisation process and characteristic the resulted polymer. The doped polypyrrole has also indicated as a prospective candidate of humidity sensor that has linear response to humidity variation ranging 30-90RH. Methanol is an highest threat of interfering

volatile chemical affecting to the polymer response to humidity.

6. Acknowledgement

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Characterization of Coconut (*Cocos nucifera L.*) Grated Residue by SEM, FTIR, TGA and XRD Analysis

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Abstract - Agricultural waste has been considered as a very important feedstock due to its renewable source and low cost materials. This study was conducted to characterize the structure of grated coconut residue left after the extraction of milk. Different chemical treatments were applied on the grated coconut residue (DCR), aiming to eliminate non-cellulosic components such as pectic substances, hemicelluloses and lignin. Alkali treatment using sodium hydroxide solubilised hemicellulose and pectin, while delignification of the fiber was done by bleaching, using acetic acid and sodium chloride. The cellulose content in DCR is 10.74%-16.91%. ATR-FTIR spectra indicated the progressive removal of non-cellulosic constituents. TGA results reveal that untreated and treated fibres were stable at temperature about 200^{o}C and decomposition of cellulose and hemicellulose occurs at temperature about 290^{o}C. SEM images of the surface morphology of the untreated and chemically treated DCR showed honeycomb like structure, and the crystallinity index of the fibers obtained from XRD analysis increases as the chemical treatments were applied on the fiber.

Keywords**:** Desiccated coconut residue, morphological structure, SEM, FTIR, TGA, XRD

1. Introduction

Sustainable development has become a priority of businesses and governments alike from ancient to date. The effects of global warming and the depletion of fossil reserves are frequently heard and it would seem to be a matter of time before people are forced to look at renewable resources to satisfy the need for materials in sustainable fashion. Science and technology are moving forward towards renewable raw materials and more environmentally friendly and sustainable resources and processes [1]. The increasing world trend towards the maximum utilization of natural resources has enhanced the study and exploration of such rich renewable natural materials. This is due to the discoverable of fall off materials that remain available as a waste product, having no industrial value and are normally incinerated or dumped without control. The byproducts of coconut, for example grated coconut residue also known as desiccated coconut residue (DCR) that is leftover after extraction of coconut milk make up a major disposal problem for the industry [4]. This waste material may be transformed into a value-added product after going through several analyses, chemical treatments and modifications. Although the DCR is easily available and can be found abundantly all over the world, the information on its utilization is still at its infancy, most probably due to the deficiency in data on its structure, morphology and components inside the DCR including cellulose. The fiber isolation from DCR poses a challenge of mixing with other impurities such as fats. In fact, it is essential to acquire the knowledge on organic and polymer chemistries as well as polysaccharides and their applications in the field of cellulose. The possibility of treating the fibers will probably broaden the fields of application for DCR. In addition, this easily available agricultural byproduct may become the main source for not only fibers but also chemicals and other industrial products.

2. Experimental

2.1 Materials and Methods

The DCR used in this study was obtained from a local store in Nilai, Negeri Sembilan, Malaysia. Several commercially available chemicals (Sigma-Aldrich co.) including sodium chlorite, sodium hydroxide and acetic acid were also used throughout the experiment. DCR was air-dried at temperature 60^{o}C for 24 hrs in an oven at until constant weight and then stored in air-tight container [4]. The proximate compositions were assayed following the AOAC method described elsewhere [11]. 5 g of ground DCR was treated with an alkali solution, NaOH (4% wt). The mixture was transferred into a round bottom flask and reflux on the heating mantle for 2 hrs, then filtered and washed several times using distilled water [7]. Following the alkali treatment, the bleaching process was completed by adding a buffer solution of acetic

acid, aqueous chlorite (1.7% wt) and distilled water at reflux for 4 hrs. The mixture was allowed to cool and filtered using excess distilled water. The bleaching process was repeated four [7].

2.2 Characterization of DCR Fiber by SEM, FTIR, TGA and XRD

The surface morphology of the untreated and chemically treated DCR were analysed using *SUPRA 55VP Field Emission Scanning Electron Microscope* (*FE SEM*) to study the surface texture and to evaluate the changes occur on the fibre surface after the chemical treatments. FTIR analyses were carried out to qualitatively identify the constituents of the grated coconut residue in untreated and chemically treated fibres. The dried fibres were finely ground, mixed with KBr and compress into pellets before analysed using ATR method. TGA was conducted on untreated and chemically treated DCR to evaluate the thermal stability of the fibres using TGA STAR^e System Mettler Toledo. Prior to the procedure, 7 to 27mg of DCR samples were pressed in 70µL aluminium oxide crucible. Then, the samples were exposed to the heat flow at temperature regime of 30^0C to 700^0C under nitrogen gas flow of 40mL/min at 10^0C/min. The heat flow into the sample will induce many physical and chemical changes which help researchers to identify and characterize the fibre. The crystalline indices of the samples were calculated from the X-ray diffraction patterns based on the following equation [8];

$X_c = I_{002} - I_{am} / I_{002} \times 100$ (Equation 1) where
X_c = crystalline index
I_{002} = peak intensity from the 002 lattice plane (22°)
I_{am} = peak intensity of the amorphous phase

3. Results and Discussion

The average values of protein, fat, ash, moisture and crude fibre content in DCR were 4.60%, 19.16%, 1.73%, 49.86% and 49.53% respectively. Chemical compositions of DCR comprises of cellulose, hemicelluloses, lignin and a small fraction of pectin and waxes. NaOH treatment and bleaching reduces the DCR weight by 80 – 90% resulting products of 10.74-16.91% cellulose fiber. The alkaline treatment solubilise the pectins and hemicelluloses compositions resulting in insoluble residue was delignified with sodium chlorite and acetic acid and completely removed hemicelluloses and lignin, resulting in almost pure cellulose fibers [2,8,10]. The fiber was bleached to obtain a whiter product with lower amounts of impurities and improved aging resistance [1].

3.1 Morphological Characterization by SEM

The morphological structure of untreated and chemically treated DCR were observed using Scanning Electron Microscope (SEM) in order to view the surface structure caused by different chemical treatments. Physically, the brown color of untreated DCR turns brownish-orange after alkaline treatment. The bleached material is obviously different and appears completely white. These color changes are due to the removal of non-cellulosic materials and other impurities upon chemical treatment of the DCR. The white color of bleached DCR gives a clear indication of almost pure cellulosic material is obtained and agrees with the chemical composition data. The physical appearances of untreated and chemically treated DCR are shown in the Figure 1.

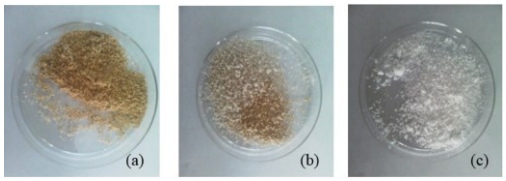

Fig. 1 Physical appearance of (a) untreated (b) alkali treated and (c) bleached DCR

The untreated and chemically treated DCR show a similar honey comb like structure (Fig. 2 a, b and c). DCR fibre still retained the honey comb like structure after the chemical treatments. This observation suggest that chemical treatments has not causes any prominent changes to the microstructure of DCR, except for the colour which was observed to become slightly bleached when compared to the original sample. A smooth cationic potential on DCR surface might be increased when oxidized by chemicals, suggesting that this fibre can be utilized as metal adsorbent. The tiny surface of bleached DCR increases the surface area, with the assumption that cellulose might be exposed on the surface due to the rupture of hemicelluloses and lignin that interconnected the cellulose fibrils [6].

SEM images shown in Figure 2 (d), (e) and (f) give indications that the fiber surface of untreated DCR are smoother and appear as long narrow fibrils compared to the alkali treated DCR. The rough and dispersed fiber surface of alkali treated DCR indicates the partial removal of the outer non-cellulosic layer composed of hemicelluloses, lignin, pectin, wax and other impurities present in the DCR. Both wax and pectin are known to surround the surface of natural fibers as a protective layer. Moreover, lignin is still preserved after alkali treatment since it forms a bridge bond with the cellulose ester and acts as a binder in the fiber components. Bleaching gives a well ordered fibril structure apparently due to the complete removal of lignin [7].

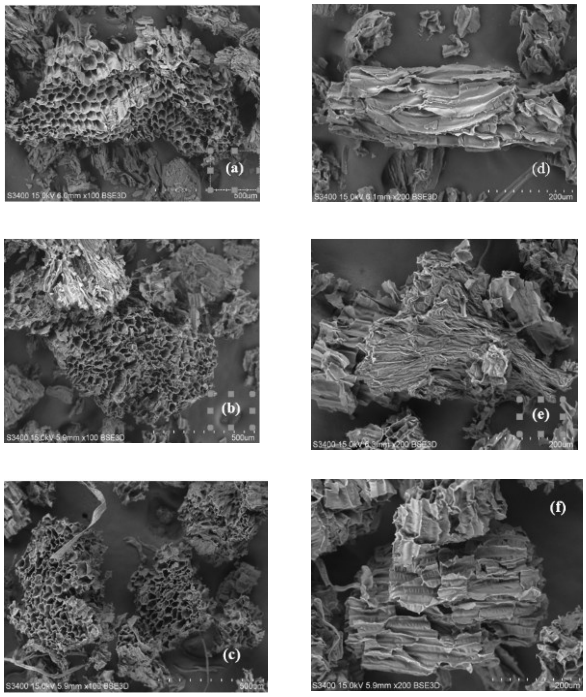

Fig. 2 Microstructures of (a) untreated (b) alkali treated (c) bleached DCR

3.2 Fourier Transforms Infrared (FTIR) Spectroscopy

Different chemical treatments induced chemical changes. The broadened bands at around 3550-3000 cm^{-1} were observed in all spectra, indicating the presence of -OH groups in the structure suggesting hydrogen-bonded stretching vibration from the cellulose and lignin structure of the fibre. The peak around 1640 cm^{-1} in all spectra corresponds to the water absorption. Both sets of peaks were ascribed to the stretching of hydrogen bonds and bending of hydroxyl (OH) groups bound to the cellulose structure. These results indicate that the cellulose component was not removed during the chemical treatment carried out on the DCR fiber [7]. The vibration at 3000-2850 cm^{-1} shows the existence of –CH groups, where the lignin and waxes were eliminated after the different chemical treatments [10]. A shoulder was observed around 1740 cm^{-1} in the spectrum of the untreated DCR. It was ascribed to the acetyl and ester groups in hemicellulose or carboxylic groups in the ferulic and p-coumeric components of lignin. The peak disappeared upon chemical treatment of the DCR indicating the removal of non-cellulosic materials. The absorption band around 1020 cm^{-1} and 800 cm^{-1} denotes the C-O stretching and C-H vibration in the cellulose structure [7]. Figure 3 below shows the ATR-FTIR overlay bands of untreated and chemically treated DCR.

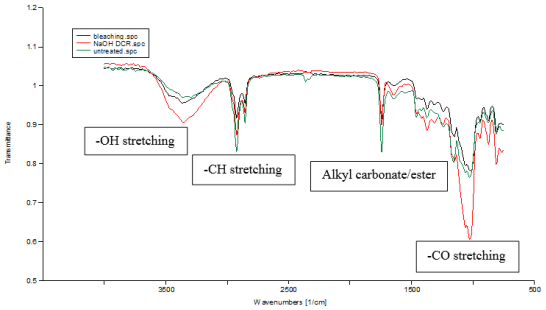

Fig. 3. ATR overlay bands of untreated and chemically treated DCR

3.3 Thermal Gravimetric Analysis (TGA)

TGA curves of untreated and chemically treated DCR were obtained during analysis in the temperature interval of 30ºC - 900ºC under the nitrogen gas flow of 10 mL/min at 10 ºC/min. TGA curves show three degradation steps for all untreated and chemically treated DCR. The first degradation curves for all samples show an initial weight loss of DCR occurring below 100ºC due to the water loss associated with moisture present in all samples. Although DCR were dried before the analysis, the total elimination of water was difficult because of hydrophilic nature of the fibers, which is present even as structurally bound water molecules [3]. All the samples were found to be stable around 150ºC - 200ºC. Bleaching process of DCR promoted an increase in thermal stability ranging 140ºC - 230ºC compared to DCR with no treatment which ranges 130ºC - 170ºC. This is due to the presence of hemicelluloses and lignin in the chemical composition of untreated DCR. These components have a lower decomposition temperature compared to cellulose and their efficient removal improves the thermal stability of the fibers. In contrast to bleaching, treatment of DCR with alkali resulted in a decrease thermal stability which was found between the temperatures ranging 150ºC - 200 ºC. The lower thermal stability of alkali treated compared to bleached DCR is attributed to the partial elimination of non-cellulosic material [7].

From 400ºC and above, all samples show considerable mass loss due to the decomposition of both cellulose and hemicelluloses in the fibers. In addition, the degradation of fibers occurs might be due to the breaking bonds of lignin present in DCR fibers [3]. Figure 4 shows the TGA curves for the untreated and chemically treated DCR.

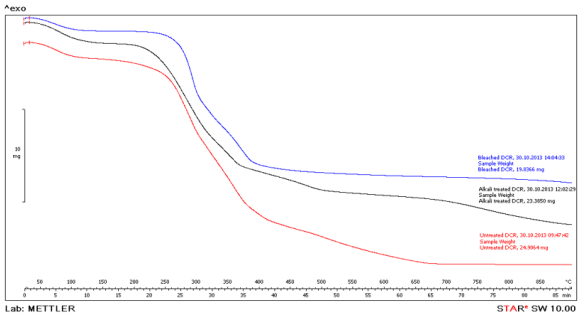

Fig. 4. TGA curves for untreated and chemically treated DCR

3.4 Crystallinity index by XRD

X-Ray Diffraction analysis was completed to evaluate the crystallinity of DCR fiber after different chemical treatments. Figure 1.5 shows the XRD patterns for untreated and chemically treated DCR. The sharp peaks can be seen at 2θ=20º and a shoulder in the region 2θ=16º. It is crystal clear that all of the samples give similar diffraction patterns. The only different concerns a slight intensity changes in the peaks, representing minor changes in the level of order in the samples. A continuous increase of the crystallinity index value was observed upon the successive chemical treatments. The alkali treatment is believed to increase in the fiber stiffness as the impurities can be removed during this treatment [7]. Bleaching shows the highest crystallinity index which was 26.50%, followed by alkali and untreated DCR which gives 10.07% and 7.35% respectively.

The increase of crystallinity index can be ascribed to the degradation caused by chemical treatments, which reduces the amorphous fraction in the natural fiber. As a result, the cellulose content is increased, taking into account that one third of the natural fiber polysaccharides are crystalline and the remaining constituents are hemicelluloses, pectin substances, or amorphous and para-crystalline regions of cellulose fibrils [5]. Cellulose is crystal in structure contrary to hemicelluloses and lignin which are amorphous in nature [7]. The crystalline structure of cellulose is due to the hydrogen bonding interactions and Van der Waals forces between adjacent molecules [9].

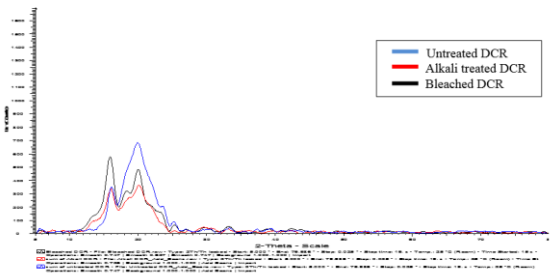

Fig. 5. XRD pattern for untreated and chemically treated DCR

4. Conclusions

The cellulose content obtained in DCR is in the range of 10.74% to 16.91%. The surface morphology of the untreated and chemically treated DCR showed honeycomb like structure. TGA studies revealed that both untreated and chemically treated DCR were stable around 200 ^{0}C, meaning this is the maximum temperature applicable for DCR and above this temperature will result in high mass loss. As the non-cellulosic components are removed, the thermal stability of DCR obtained from TGA analysis and crystallinity index measured using XRD increases showing that cellulose is high in strength and crystal in nature. These characteristics is necessary to support better understanding of DCR structure-property correlations and hopefully provide better opportunity in bringing the existing coconut industry to the higher level leading to better utilization of DCR in the future as part of the global vision towards a bio-based economy.

Acknowledgment

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Carbohydrate and Protein Interaction in Edible Film Production by Extruder as Mixing Unit and Compression Molder as Molding Unit

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Abstract - In edible film production by compression molder, preparing uniform dough is one of major problem that should be overcome. Extruder already widely use as mixing unit in plastic production that will be used in this research by some modification. This research was studied carbohydrate and protein interaction during preparation of dough in edible film making by extruder as mixing unit and compresion molder as molding unit. Tapioca would be used as source of carbohydrate and Soy Protein Concentrate (SPC) would be used as source of protein. The concentration of SPC was varied 0%, 20%, 40%, 60%, 80% and 100%. Increasing of SPC concentration would increase Water Holding Capacity of dough. Dough's texture would increase if SPC concentration increased from 0% to 20% but decreased if concentration increased from 30% to 100%. Result from Rapid Visco Analyzer study showed that increasing of SPC concentration would decrease final viscosity and increase peak time. Microstructure analysis by Scanning Electron Mycrograph showed that during mixing, protein would perform network like fiber and part of tapioca granule break. After mixing by extruder, the dough would be mould by compression molding to produce edible film whereas increasing of SPC concentration would increase tensile strength and solubility of edible film but decreased film's solubility .

Key words : tapioca, Soy Protein Concentrate, dough, Rapid Visco Analyzer, Scanning Electron Micrograph.

1. Introduction

Research on edible films and coatings in foods is partly driven by industry due to high demand of consumers for longer shelf-life and better quality of foods as well as environmentally friendly materials. Edible films are generally formed from hydrophilic components such as proteins or polysaccharides. Biopolymer films, which contain both protein and polysaccharide ingredients, may advantageously use the distinct functional characteristic of film-forming ingredients. Soy Protein and tapioca already widely used as edible film's based material (Brandenburg *et al.,* 1993; Gennadios *et al.,* 1993; Choi *et al.*, 2003; Marseno dkk. 1999; Harris 2001; Bergo *et al.*, 2007; Chillo *et al.*, 2008; Perez *et al.*, 2009)). This research used Soy Protein Concentrate (SPC) and tapioca as edible film based component. Previous study (Lindriati *et al.* ,2007a; 2007b), showed that *edible film* from protein combined with carbohydrate could produce better physical and mechanical properties than protein or carbohydrate alone.

Much of the research into edible films has involved the production of films from the method of solvent casting. There are many researches try to develop thermal processing methods such as compression molding and extrusion (Cunningham et al., 2000; Sothornvit et al., 2003; Pommet et al., 2003; Sothornvit et al., 2007; Hernandez-Izquierdo et al., 2007; Guerrero et al., 2010). Extrusion is an attractive alternative method for protein and polysaccharide film formation, because its speeds processing and requires less space compare to the traditional solution-casting method. Generally, compression molding of sheet is studied as a precursor to extrusion, in order to demonstrate material flowability and fusion and identify conditions suitable for extrusion (Sothornvit et al., 2007).

Previous study showed that before molded based material must be mixed intensifly to let water and glycerol molecules penentrated in to carbohydrate and protein macromolecule otherwise we could not produce a transparant films. Lindriati and Arbiantara (2009) used mortar porcelain to mix edible film based material and aged the dough for one week to produce transparant and flexible film but at least one night aging still needed. Extruder already widely used in plastic industry as intensif mixing unit. This research would use extruder that modified for intensif mixing unit in preparing dough of SPC and tapioca before molded by compression molder. This research objective was to study effect of SPC : tapioca ratio on dough and edible film properties. Dough was prepared by single screw extruder and edible film produced by compression molder.

2. Material and Method

2.1 Material

SPC powder was obtained from local market (industrial grade). The composition of the SPC powder was reported as 76% protein, 19% carbohydrate, 4% and 1% ash. Tapioca starch was obtained from local market. The composition of tapioca was reported as 4% protein, 93% carbohydrate, 2% fat and 1% ash. Glycerol was used as plasticizer that was p.a grade (merck).

2.2 Dough formation

Mixtures of SPC powder and tapioca (100 g) with addition of glycerol (20% v/w) and deionized water (70% v/w) were mixed in a single screw extruder. The proportion of ISP:tapioca was varied (0:100, 10:90, 20:80, 30:70, 40:60, 50:50). All materials were thoroughly blended with single screw extruder at room temperature and operated at speed of 100 rpm. The dough then thermally compacted using a prototype *compression molding*.

A prototype modular single screw extruder with L/D ratio of 1:5 and a barrel diameter (D) of 30 mm was used. The channel depth (H) in the metering zone has 2 mm, while in the feed section were used 5 mm (compression ratios-CR- of 2.5) as shown in figure 1. The axial length of feed, compression and metering sections (L_1, L_2 and L_3) are the same and equal to 150 mm.

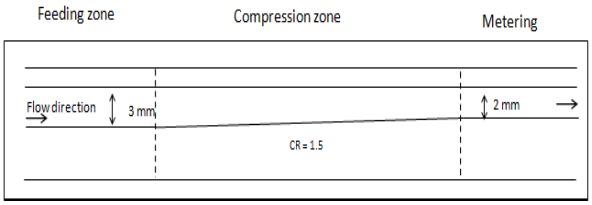

Figure 1. Screw geometry

2.3 Edible film formation

About 10 gram of dough that was prepared with single screw extruder was placed between two sheets of aluminium (0,2 mm thick and 100 cm^2) and then thermally compacted using a prototype compression molder (Figure 2)

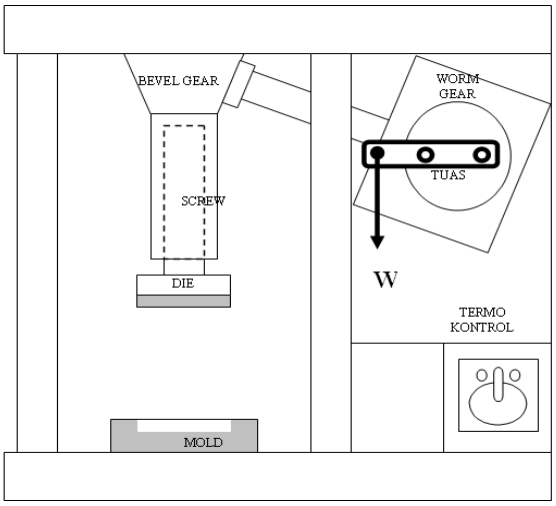

Figure 2. Prototype of compression molder

The compression molder was operated at 150°C, a pressure of 1,1 Mpa was applied for 10 min. The aluminium sheets were allowed to cool for 3 min before removing films samples. The films were kept in to a chamber that already conditioned with silica gel.

2.4 Dough Characterization

Water Holding Capacity (WHC) (AACC, 1991, modified)

An empty centrifuge tubes were dried and weighed (a gram). WHC measurement begined by weighing 0.5 grams of sample (b g) and inserted into the centrifuge tube. Distilled water as much as 7 x weight of sample was added. Sample mixed by vortex and than centrifuge for 5 min at 2000 rpm. Supernatant was decanted and precipitate weighed (c g). WHC calculated by :

$$\%WHC = \frac{(c-a)-b}{b} x100\%$$

a = weight of empty centrifuge tube; b = weight of sample; c = weight of the sample and tube after centrifuged

Texture

Penetrometer was used for texture measurement. 25 gram of dough samples were weighing and formed round and texture measurement done on 5 different points. The results of measurement was expressed as average in mm/sec.

Digital Viscosity Measurement (RVA, New Scientific)

3 gr of sample (dry basis) was added by water and weighing until 25 gr. 15 minutes If not dissolve completely may be repeated until dissolved. Lift the lever RVA, attach cans in place. Termocline program select: collect, then auto for existing programs be set tool. Select the desired method. Select run, auto, select

auto, mode to run, wait until the equipment is ready to operate (press lever down RVA).

Scanning Electron Microscopy (SEM, Chuang and Yeh, 2004)

Samples previously dried by freeze drier to achieve a moisture content of less than 5%. Dried samples sprinkled on the stub and then coated with gold dust. Samples that have been covered with gold included in the machine for SEM observation at a magnification of 2000x.

2.5 Film characterization

Tensile strength and elongation (according to ASTM standards D638-94 in Chang et al, 2000).

Pieces of edible film with a width of 10 mm and a length of 80 mm may be stored in a jar containing silica gel for one day. Then the tensile strength was measured using a Universal Testing Machine (Shimadzu). The size of the specimen can be seen in Figure 3

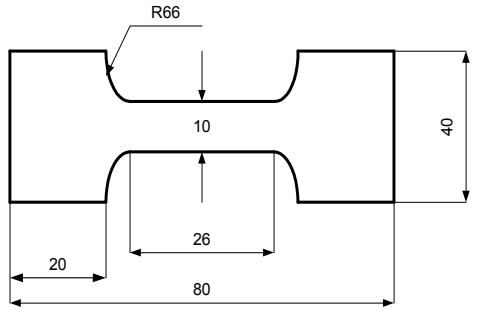

Figure 3. Tensile Test Specimens (according to ASTM D638-94 in Chang et al, 2000).

The tensile strength can be calculated with this formula:

$$\sigma = \frac{F}{A}$$

σ = tensile strength (N / mm 2); F = tensile force (N); A = area of work (mm2).

In accordance with tensile strength procedure, strain measurement was done using Universal Testing Machine. The strain can be formulated:

$$\varepsilon = \frac{\Delta l}{l_0}$$

ε = Strain (%); Δl = length addition (mm); l_0 = Initial length (mm).

Solubility of edible films (Gontard et al., 1992).

Measurement of solubility in water of edible film based on the percentage of initial dry material that dissolves. Pieces of edible film with a size of 2.5 x 5 cm were dried in an oven 105^{o}C for 24 hours, then weighed (a gram). After drying edible film was soaked in 30 ml of distilled water and placed in a container that was covered with plastic and then stored at room temperature for 24 hours. Pick up the resembled pieces and then dried in an oven at 105^{o}C for 24 (b gram). Solubility value is g/g calculated by (a-b)/a or ((a-b)/a) x 100% if the units was % .

3. Result and discussion

3.1 Water Holding Capacity (WHC) of Dough

The results of WHC measurement on dough after mixing with extruder with variation of the SPC : tapioca ratio were ranged between 0.233% - 2.496%. WHC lowest value obtained from SPC: tapioca ratio 0: 100, while the highest WHC values obtained from SPC : tapioca ratio 50:50. The addition of SPC had significant effect on WHC value ($p < 0.05$). The effect of ISP: tapioca ratio on dough WHC values are presented in Figure 4.

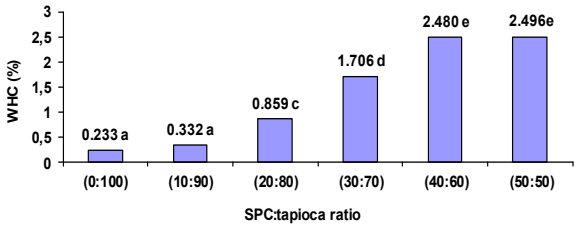

Figure 4. Effect of SPC : tapioca ratio on dough's WHC

Increasing of SPC ratio would increase WHC value (Figure 4). Tendency of SPC protein to bound water higher than tapioca. According to Hansen (1978), increasing tendency of dough to bound water depend on protein content. According to Kinsella (1984), increasing in water absorption due to the addition of protein usually followed exponential function. The amount of water retained by the protein depend on composition of amino, exposed polar groups, conformation and hydrophobicity as well as processing conditions. SPC was one kind of protein that had high water binding capability.

3.2 Texture of Dough

The results of texture measurement on dough after mixing with extruder with variation of SPC : tapioca ratio were ranged between 2,49 – 4,23 mm/second. WHC lowest value obtained from SPC : tapioca ratio 50:50, while the highest WHC values obtained from SPC : tapioca ratio 20:80. The addition of SPC had significant effect on texture value ($p < 0.05$). The effect of ISP: tapioca ratio on dough texture are presented in Figure 4.

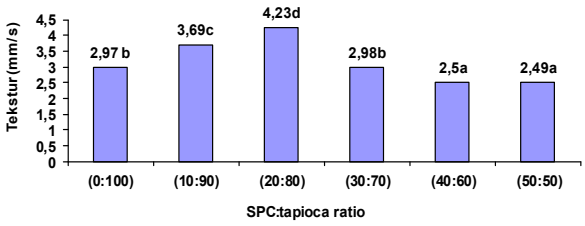

Figure 4. Effect of SPC: tapioca ratio on dough's texture.

Increasing of SPC : tapioca ratio from 0:100 to 20:80 increased texture value. Increasing of SPC : tapioca from 20:80 to 50:50 decreased texture value. Decreasing of texture because of increasing SPC ratio from 40:60 to 50:50 were not significantly different ($p > 0.05$) (Figure 4).

According to Maurice and Stanley (1978) and Sheard et al. (1984) increased protein addition would significantly affected material's rheology. During extrusion, protein would perform network protein-protein that had structure fibers like. According to Noguchi (1989) there were differences in the structure of protein's network that extruded at high moisture content (above 35%) with low moisture content (20-25%). The network performed at low moisture content was not a compact fiber, but a aggregates that was expands and spongy. Extrusion at high moisture content would produce protein-protein network that was compact like fiber. The important thing about the role of water in extrusion were in the formation of protein-protein network structure.

SPC addition less than 20% (SPC : tapioca ratio 20:80) improved value of texture test it means the dough getting softer (Figure 4), This is probably because effect of water absorbtion by carbohydrate. High ratio of carbohydrate could make large amount of water absorb in the carbohydrate matrix and water that was absorbed by protein decreased. This would resulted in formation of soft and spongy protein-protein network.

SPC addition more than 20% (SPC : tapioca ratio 20:80) decreased value of texture test it means the dough getting harder (Figure 4). This is probably due to the amount of water absorbed in protein matrix was sufficient to give the effect of extrusion wit h high moisture content. According to Noguchi (1989), at high moisture content protein-protein network would perform fibers and fibrous like structure that could cause the dough more compact.

3.3 *Rapid Visco Analyser* (RVA) of dough

The results of the RVA analysis on dough's pasting characters in this study could been seen in Table 1 and Figure 5

Tabel 1. RVA analysis on dough of edible film preparing by single screw extruder with SPC : tapioca ratio.

Ratio of ISP : tapioka	*Final viscosity* (cp)	*Peak time* (minute)
0 : 100	1391	4,20
10 : 90	1186	4,33
20 : 80	1182	4,75
30 : 70	1110	4,53
40 : 60	1084	4,67
50 : 50	709	6,60

The result of RVA analysis indicated that increasing of SPC ratio would decrease viscosity and increase peak time value (Table 1). According Sandstedt and Abbott (1961), the character of starch pasting influenced by starch concentration, so in this study increasing of SPC ratio would decreaces starch concentration that would decrease viscosity and increase in peak time. The presence of heat and pressure during extrusion process would denaturate protein and perform protein-protein network, complex structure of protein could decrease viscosity of dough's paste. Lin et al. (2000) reported that the formation of large molecular weight from soy protein during extrusion process could result decreasing of protein solubility.

The greatest reduction in viscosity value was on increased SPC ratio from 40:60 to 50:50 as well as increased in peak time. This was probably due to increase of network protein – protein that formed. Formation of starch paste would be interrupted by formation of protein-protein network.

According to Coughlan et al. (2000) and Zaleska et al. (2000), formation of protein-carbohydrate complex would increase functional properties of gel such as viscosity. In this study, viscosity of gel decreased in increasing protein addition. Probably in this study interaction between carbohydrate - protein was not forming new covalent or ionic bonds to form complex.

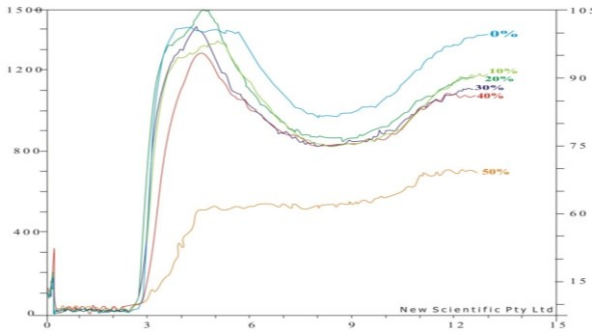

Figure 5. Chart result of RVA analysis of edible film's dough materials with variation of SPC : tapioca ratio after mixing with a single screw extruder.

3.4 Scanning Electron Micrograph (SEM) of dough

Microstructural analysis by SEM performed at 2,000 x magnification presented at Figure 6, Figure 7 and Figure 8.

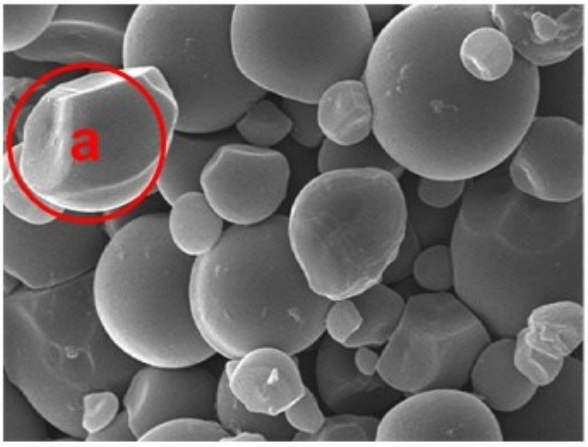

Figure 6. SEM micrographs of edible film's dough at SPC : tapioca ratio 0 : 100 (a = the ruptured starch granules).

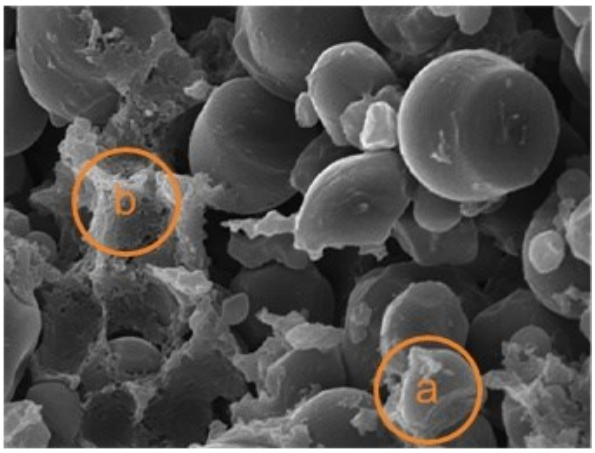

Figure 7. SEM micrographs of edible film's dough at SPC : tapioca ratio 30 : 70 (a = the ruptured starch granules ; b = fiber like protein-protein network).

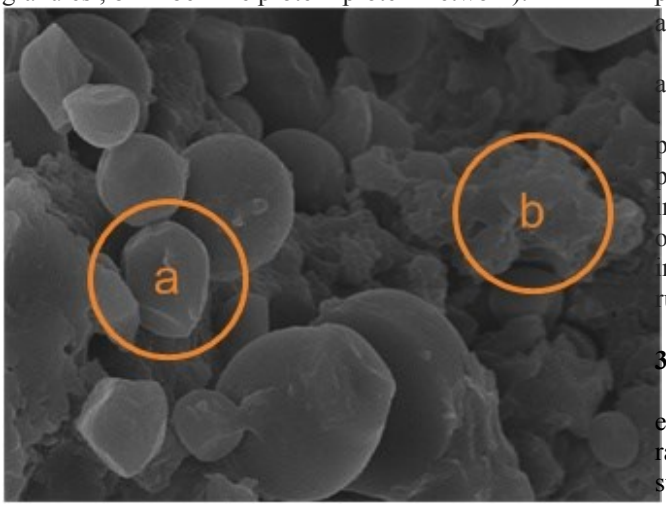

Figure 8. SEM micrographs of edible film's dough at SPC : tapioca ratio 50 : 50 (a = the ruptured starch granules ; b = fiber like protein-protein network)

Figure 6 was a micrograph of edible film's dough with SPC: tapioca ratio at 0 : 100. The image showed existence of intact and ruptured starch granules. Several studies (Molina-Garcia et al., 2007 and Shariffa et al., 2009) showed there were intact and ruptured starch granule after treatment as showed at Figure 6. Granule size in this study was bigger than these studies, it was presumably because the effect of shear stress during extruder mixing. It was showed at figure 6 that starch granule was not melted nor gelatinized.

According to Baud et al. (1999) a large molecule will be broken during extrusion. Meanwhile, Barron et al. (2001) studied that heat treatment and shear forces could result in variety of physical and chemical changes, where the structure starch granules damaged, natural crystals melted and stability of macromolecules disturbed. In this study starch granules was not melted because the screw design and operating conditions was not sufficient to destruct and melt starch crystals.

Figure 7 and Figure 8 were micrographs of edible film's dough with ISP: tapioca ratio at 30:70 and 50:50. It were showed presence of aggregates resembling fibers, which supposedly as protein-protein network. Chiang (2007) studied artificial meat composed from SPC and tapioca produce by extrution, SEM observation showed presence of fiber like structure that was protein-protein network, whereas extrusion operated at temperatures above 130^{o}C and the fibers formed were more compact. The results of this study indicated that although extrusion was operated without heating the fibers still formed due to influence of shear stress.

According to de Mesa et al. (2009) in artificial meat formation, network protein occurs on 15% SPC addition. Li et al. (2010) studied that network protein formed on the addition of SPC 30%. During extrution, shear stress and thermal denaturation resulted in aggregates such as fibers which was composed of proteins crosslinking and interactions between proteins and other components (Sheard et al., 1984)

Figure 6, Figure 7, Figure 8 showed that the amount of starch ruptured increased on inceasing SPC : tapioca ratio from 0 : 100; 30:70 and 50:50. This was presumable because formation of fibers like protein-protein net work which could increase shear forces and increased starch granule degradation. Design and operating conditions of extruder in this study resulted in protein to form fiber like network and resembling rupture of starch granules.

3.5 Tensile Strength of edible film

The results of tensile strength measurement on edible film with variation of SPC : tapioca ratio were ranged between 0,179 Mpa – 0,598 Mpa. Tensile strength lowest value obtained from SPC : tapioca

ratio 0:100, while the highest tensile strength values obtained from SPC : tapioca ratio 30:70. The addition of SPC had significant effect on tensile strength value ($p < 0.05$). The effect of SPC : tapioca ratio on edible film tensile strength are presented in Figure 9.

Figure 9 showed that, in general, increasing SPC ratio would increased tensile strength values. Lindriati et al. (2007b), studied effect of protein from Jack bean (*Canavalia Ensiformis*) addition on the matrix of edible film from cornstarch. The results showed that increasing of protein addition would increase tensile strength, but the addition of the protein more than 20% influence on increasing tensile strength was not significantly different ($p < 0.05$). Poeloengasih and Marseno (2003), showed a similar trend in which the tensile strength of edible films from tapioca starch would increase with the addition of winged bean proteins. Coughlan et al. (2004) studied addition of polysaccharides on the edible films from whey protein, the result showed decreasing of tensile strength due to the addition of polysaccharides.

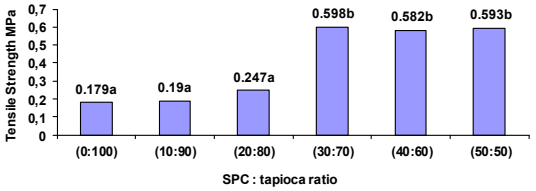

Figure 9. Influence of SPC: tapioca ratio to the value of the tensile strength of edible film.

According to Guilbert and Graille (1994), generally mechanical properties of edible films from protein better than from starch. This is because starch is a homopolymer, whereas the protein has a specific structure composed at least 20 hydrogen bonds from different monomers. This resulted in functional properties of proteins more variable, especially in their ability to form intermolecular bonds. So addition of protein fractions in the matrix of edible film from carbohydrates could increase tensile strength.

Tensile strength increased significantly ($p < 0,05$) when the ratio of SPC : tapioca increased 20 : 80 to 30:70. This is supported by the data of dough texture measurements (Figure 4, where a significant decrease ($p < 0.05$) of texture value when the ratio of SPC : tapioca increased from 20:80 to 30:70. This was probably due to the formation of protein network at 30% SPC addition, the amount of water absorbed by protein was sufficient to give effect of extrusion with high moisture content. According to Noguchi (1989) that could produce fibers like protein-protein network that resuled in a more compact dough.

3.6 Elongation of edible film

The results of elongation measurement on edible film with variation of SPC : tapioca ratio were ranged between 22,756% - 157,247%. Lowest value of elongation obtained from SPC : tapioca 50:50 and highest elongation value obtained from SPC : tapioca ratio 0:100. The addition of SPC had significant effect on tensile strength value ($p < 0.05$). The effect of SPC : tapioca ratio on edible film

SPC addition on edible film base material could reduce elongation value (Figure 10), which means decreasing elasticity. Lindriati et al (2007b) showed similar result where addition of protein from Cannavalia Ensiformis to the matrix of edible film from maizena decreased elongation. Research of Poeloengasih and Marseno (2003), also showed a similar trend, where the addition of protein fractions decreased elongation of edible film from tapioca starch.

The addition of protein fractions in the matrix of starch edible film could increase number of intramolecular hydrogen bonds. This could reduce the ability of the plasticizer in reducing the strength of hydrogen bonds. According to Choi and Han (2001), plasticizers can infiltrate between intramolecular hydrogen bonds thereby increasing the distance between adjacent chains which can reduce the strength of hydrogen bonds and can ultimately enhance the flexibility and extensibility of edible film. In addition, during mixing with extruder, protein formed network that likely fibers due to shear and heat generated, when dough was molded by compression molder protein-protein network would strengthen edible film matrix but make the film less elastic.

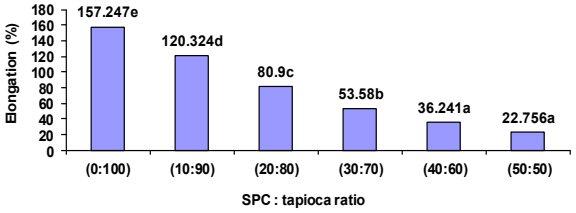

Figure 10. Influence of SPC : tapioca ratio to elongation of edible film.

3.7 Solubility Edible Films

The results of solubility measurement on edible film with variation of SPC : tapioca ratio were ranged between 22,756% - 157,247%. Lowest value of elongation obtained from SPC : tapioca 50:50 and highest elongation value obtained from SPC : tapioca ratio 0:100. The addition of SPC had significant effect on solubility value ($p < 0.05$). The effect of SPC : tapioca ratio on edible film solubility presented on figure 11.

Generally, increasing SPC ratio would decrease solubility value. This was probably due to formation of fibers like protein-protein network. Jeunink and Cheftel (1979) and Noguchi et al. (1989), conducted a study on artificial meat with ISP addition, where

solubility of artificial meat decreased when compared to the basic material because of formation of protein - protein network presence by heat and shear forces. Lin et al. (2000) reported the formation of large molecular weight of soy protein during extrusion process could resulted in decreased of protein solubility.

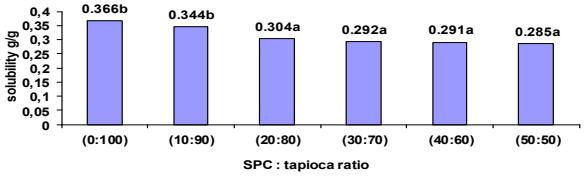

Figure 11. Influence SPC : tapioca ratio to solubility value of edible film

4. Conclusion

Ratio of SPC : tapioca on edible film affect the value of WHC and texture of dough as well as affect on tensile strength, elongation and solubility of edible film. Increasing SPC ratio would increase WHC of dough and edible film's tensile strength but decrease elongation and solubility. At the SPC ratio from 0:100 to 20:80 texture would increase but would decrease when SPC ratio increased from 30:70 to 50:50. The result of RVA analysis indicated that increasing of SPC ratio would decrease viscosity and increase peak time value. The observation of SEM micrographs showed that the changes that occur in the presence of shear forces caused by the screw i study resulted in protein to form fiber like network and resembling rupture of starch granules.

Ratio of SPC : tapioca that produced edible film with the highest tensile strength (0.598 MPa), elongation (53.580%) and lowest solubility (0.292) was 30:70. Combination of single screw extruder as mixing unit and compression molding as molder unit could be applied in edible film production.

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Drying Characteristics of Sweet Corn Milk in a Moving Bed of Inert Particles

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Abstract - Drying characteristics of sweet corn milk in a vortex-like moving bed of inert particles was studied in this work in terms of the product moisture content, specific water evaporation rate, product recovery and drying efficiency. These parameters were examined for different experimental variables, i.e., the inlet air temperature (100-120°C), airflow rate (245-323 m^3/h), feed rate (0.6-1.5 kg/h) and mass of inert particles (0.7–1.3 kg). The initial moisture content of sweet corn milk in this experiment was found between 91 and 95% on wet basis. The results showed that the designated experiments could produce sweet corn milk powders containing moisture of about 3-12% on wet basis depending upon the operating conditions. The specific water evaporation rate during drying process correlated linearly with the mass of inert particles, inlet air temperature, and airflow rate. The product recovery of sweet corn milk powder that properly collected from drying unit varied from 60 to 89% (dry basis). The efficiency of drying process ranged between 6 and 48% and was affected significantly by the mass of inert particles and inlet air temperature.

1. Introduction

Sweet corn is becoming popular in Indonesia due to its unique properties such as sweet and soft kernels. This is utilized mostly in fresh form that applied to various vegetable recipes or consumed as corn on the cob, e.g., a steamed corn or a grilled corn. Unluckily, doing a business on this product seems to gain less economic value added because it is less appreciated by the consumers who require a newly innovative and impressive product. Several studies had been done earlier to work out this problem through development of various beverages using sweet corn kernels such as sweet corn milk [1], fortified sweet corn and soybeans milk [2], and the mixed sweet corn and mungbean milk [3]. They proved that such products are likely getting more benefits since getting a very good interest from the consumers. This condition has triggered the present study to carry out a further development on these results by producing sweet corn milk powder that can serve either as the instant beverages or as the ingredient in a variety of human food menu. Under this circumstance, this product will get additional advantages, i.e, simplified fortification with other products, an increased shelf life, and a reduced packaging and transportation costs.

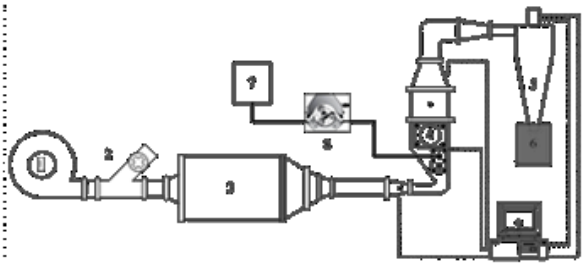
A key success in the production of the instant food powder is determined strongly by drying process that will be inappropriate to be accomplished using a conventional method, e.g., oven drying, because it needs a prolonged process, reduces the product color quality and results in low drying efficiency. The spray and drum drying methods are commonly selected to complete these purposes, but still suffer from high capital and running costs. Hence, an attempt had been made through this study to overcome some of these limitations by introducing an alternative drying, which known as a spouted-vortex-bed (SVB) drying method. The SVB drying system exploits the fluidization of inert particles, i.e., Teflon pellets, as the drying means of food liquids in a gas-particle contacting chamber. Several benefits such as a high thermal efficiency and a relatively inexpensive construction costs cause the SVB system comparable to spray and drum dryers [4]. However, there are few studies found in relevance journals regarding the sweet corn milk drying in a continuously moving bed of inert particles subjected to vortex-like motion. For that reason, the main objective of the present study was to investigate the effects of experimental variables such as inlet air temperatures, airflow rates and feed rates, and mass of inert particles on the drying characteristics of sweet corn milk in experimental drying setup in terms of product moisture content, the specific water evaporation rate, product recovery and drying efficiency. The information on sweet corn milk powder quality produced by this study can be found in separated publication [5].

2. Materials and Methods

2.1 Experimental Drying Setup

It is shown in Figure 1 that an experimental drying setup is composed of five main subsystems namely the drying chamber, electrical heater, air blower, feeder, and cyclone separator. This drying system was a result of modifications on available SVB dryer developed in former study [9] particularly in terms of electrical heater and feeder subsystems. A drying chamber was built using ±1.2 mm thickness of

stainless steel sheet with volume of about 2450 cm^3. The bottom section shape of a chamber was constructed similar to a half-hopper with an inclined wall angle was around 60° to the horizontal. The flow of heated air from electrical heater entered tangentially into a drying chamber via rectangular channel with cross-sectional area of about 18 x 127 mm^2. The rest profile of an upper side of a chamber was likely a box shape with a cavity cross-sectional area and height of approximately 133 cm^2 and 105 mm, respectively. Two circular glass windows were set on the wall of drying chamber to facilitate the inspection of a moving bed of inert particles during drying process. Teflon pellets of cubical in form and roughly with size of about 5 mm was used as the inert particles due to its distinctive properties, i.e, a very high temperature resistance and non sticky surface. These pellets were continuously fluidized by tangential airflow through the inlet near the bottom part of a drying chamber to impart a vortex-like motion. Ambient air was heated by six 1.5 kW electrical coils that arranged in three parallel circuits and transferred to a drying chamber through a 9-cm diameter steel pipe by a radial blower (KATSU Model ZYJD-750). The temperature and hot airflow rate in drying system were regulated by a thermostat controller (TEW Model IL-80EN) and an exhausting valve, respectively. A dosing pump (Chem-Tech 100/150) was used to supply sweet corn milk into the drying chamber to come in contact with moving bed of inert particles. The variation of feed rates could be regulated using a volumetric speed controller found in a dosing pump. Sweet corn milk powder released from a drying chamber were collected in a cyclone separator and transferred to the plastic bags.



1. radial blower
2. airflow rate regulator
3. electrical air heater
4. drying chamber
5. cyclone separator
6. product collector
7. feed container
8. dosing pump
9. data logger

Figure 1. Experimental SVB Drying System

2.2 Preparation of Sweet Corn Milk

Raw sweet corn milk was prepared from the sweet corn kernels of "Bisi Sweet" variety using the method, developed by Prabhavat et al. [7]. The kernels were initially washed and steamed at ±100°C for about 15 minutes. After draining and rinsing, the softened sweet corn kernels were extracted with distilled water in a milling unit. The required proportions of sweet corn kernels to water were 1:2 during this milling process. The obtaining slurry was then separated into aqueous suspension (sweet corn milk) and fibrous byproduct by a centrifugal separator. The sweet corn milk was then filtered through a double folded cotton fabric to ensure the removal of all solid particles. The moisture content of these sweet corn milks varied between 91 and 95% on wet basis.

2.3 Procedures of Drying Experiment

An experimental SVB dryer was firstly allowed to run for about 25 minutes to attain the steady-state operating conditions. About 500 ml sweet corn milk was then fed into a drying chamber using a dosing pump that controlled automatically the feeding rate. Drying process started when sweet corn milk coming into contact with inert particles in a drying chamber and ended once all obtaining powdery dry product was released from cyclone separator. During experiments, the inlet, inside, and outlet temperatures of a drying chamber were monitored and continuously recorded. In addition, the data recording was also done for temperatures of ambient, feed and dry product. The remaining mass of sweet corn milk that accumulated in drying setup was noted also to enable calculations of heat and moisture balance during drying process.

2.4 Parameters of Drying Performance

The dryer performance was evaluated in terms of the final product moisture content, specific water evaporation rate, product recovery and drying efficiency. The moisture content of sweet corn milk powder (X_f, decimal) was determined using AOAC method [8]. The specific water evaporation rate (W_s, kg $H_2O/h.m^3$) represents the ability of dryer to evaporate moisture from sweet corn milk for given operating conditions. Evaluation of W_s values was based on the overall heat balance and determined using Eq. 1 as described in Taruna and Jindal [6]

$$W_s = \frac{1}{V_c} \frac{M_a C_a (T_i - T_o) - H_s}{\left[\frac{1 - X_o}{X_o}\right] C_d (T_o - T_a) + C_w (T_o - T_a) + \lambda} \quad (1)$$

where, V_c = drying chamber volume (m^3), M_a = mass flow rate of drying air (kg/h), C_a = specific heat of air (kJ/kg.°C), T_i = inlet air temperature (°C), T_o = outlet air temperature (°C), H_s = heat losses (kJ/h), X_o = initial moisture content of sweet corn milk (decimal), C_d = specific heat of dry matter (kJ/kg.°C), T_a = ambient temperature (°C), λ = latent heat of water evaporation (kJ/kg) and C_w = specific heat of water (kJ/kg.°C). The product recovery was measured as the ratio of total solids in dry product to the total solids in the feed on percentage basis. Drying efficiency (η_d) is the ratio of the heat of hot air utilized for drying to the

theoretical (maximum) available heat of hot air for drying. Considering the mass balance of water and the heat balance across the bed, the value of drying efficiency (η_d) can be computed using Eq. 2 as suggested by Kato et al. [10].

$$\eta_d = \left[\frac{T_i - T_o}{T_i - T_{wi}}\right] x100\% \qquad (2)$$

where, T_{wi} = inlet wet bulb air temperature (°C).

2.5 Operating Conditions and Data Analysis

The effect of operating conditions on the drying performance was examined at least two replications for each designated drying test. The drying air temperature was measured at the inlet part of the drying chamber using calibrated thermocouples. A relatively very high inlet air temperature (T_i) of 100, 110, and 120°C was used during drying experiments to overcome high moisture content of raw sweet corn milk. Airflow rate (Q) was operated at 245, 285, and 323 m^3/h in order to enable the agitation of inert particles and a steady state drying conditions. The feed rate of sweet corn milk (F) was varied in three levels of 0.6, 1.0, and 1.5 kg/h, whereas the amount inert particles (m) were set at 0.7, 1.0 and 1.3 kg in conjunction with other drying operation parameters. The software package of SPSS 14.0 (evaluation version) was used to determine descriptive statistic, histogram and to analyze the correlation between the experimental variables on the selected dryer performance parameters at $p \leq 0.05$.

3. Results and Discussions

The correlation coefficients (r) between the dryer performance and the SVB drying conditions are presented in Table 1 based on eighty-one experimental runs. It appears that the mass of inert particles (m) and inlet air temperature (T_i) were dominant to influence the SVB dryer performances as compared to other experimental variables.

Tabel 1: Correlation coefficients (r) between experimental variables and SVB dryer performance parameters

Parameter	Experimental variables			
	T_i	F	Q	m
Xf	-0.522 *	0.364 *	-0.163	-0.440 *
Ws	0.459 *	-0.196 *	0.206 *	0.631 *
PR	0.286 *	-0.289 *	0.124	0.718 *
ηd	0.416 *	-0.211 *	0.202 *	0.650 *

* Significant ($p \leq 0.05$)

These r values that were statistically significant provided important information on the effect of drying conditions on the parameters of dryer performance as discussed in the following sections.

3.1 Product Moisture Content

Fresh sweet corn milk could be dehydrated adequately using experimental setup from an initial moisture content of about 91-95% wet basis to a final moisture content ranging from 3 to 12 % wet basis depending upon the operating conditions. Figure 2 shows the distribution of the moisture content of dry sweet corn milk products. For designated operating conditions, the resulting sweet corn milk powder that consisted of moisture less than 8% (wet basis) reached approximately 75% of the number of samples obtained in the present study.

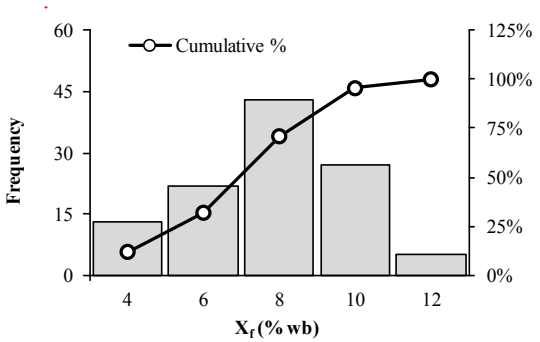


Figure 2. Distribution of moisture content of sweet corn milk powder (X_f) obtained from a variety of drying experiments.

It can be seen in Figure 3 that the dry sweet corn milk samples with moisture content less than 8% on wet basis were obtained mostly when the inlet air temperature were operated between 100-120°C while the mass of inert particles were set at 1.3 kg. Table 1 confirms that increased values of inlet air temperature and mass of inert particles decreased significantly moisture content of sweet corn milk powder samples. An increase in the inlet air temperature and mass of inert particles improved progressively the ability of drying air to evaporate water from fresh sweet corn milk since the heat supplied and contact surface area are greater than before and thus enable the reduction of moisture content of final product. It was not feasible to

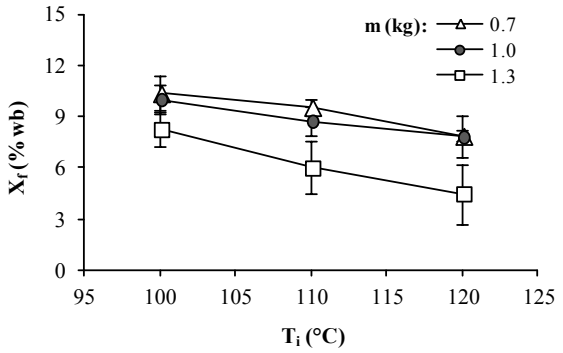


Figure 3. Relationship between product moisture content (X_f) and inlet temperature (Ti) for various mass of inert particles (m) when running at F = 1,5 kg/h and Q = 323 m^3/h

dry sweet corn milk using high feed rate more than 1.5 kg/h when operating the inlet air temperatures about 100°C and Teflon pellets about 0.7 kg because it resulted in a highly product moisture content and increased accumulation of agglomerated sweet corn milk inside a drying chamber. This might occur due to insufficient provision of heat and contact surface area of heat by the inert particles. However, the present work indicated that the selected experimental variables applied in the drying experiments could produce instant sweet corn milk powder with moisture content for safe preservation.

3.2 Specific Water Evaporation Rate

The specific water evaporation rate (W_s) during drying of sweet corn milk is shown in Figure 4 as a function of inlet air temperature (T_i) for various mass of inert particles (m) and the selected experimental conditions. The trend lines displayed in Figure 4 are supported by the linear correlation analysis presented in Table 1, indicating a correlation coefficient (r) of about 0.459 between specific water evaporation and inlet air temperature. The most significant factors affecting the specific water evaporation rate was actually the mass of Teflon pellets (r = 0.631). The specific water evaporation rate increased with an increase in the Teflon pellets mass from 0.7 to 1.3 kg, but appeared to show no differences when it was set from 0.7 to 1.0 kg. Increased mass of Teflon pellets might greatly increase the surface area for the heat transfer to facilitate better use of heat for product moisture evaporation. The other two significant factors affecting the specific water evaporation rate included the feed rate (r = -0.196) and mass airflow rate (r = 0.206). An increase in the feed rate decreased the specific water evaporation rate, indicating that the heat supplied to the system could not be further utilized to dry the sweet corn milk. Under this condition, it is suggested to increase the application of inlet air temperature and the amount of inert particles to avoid a failure process.

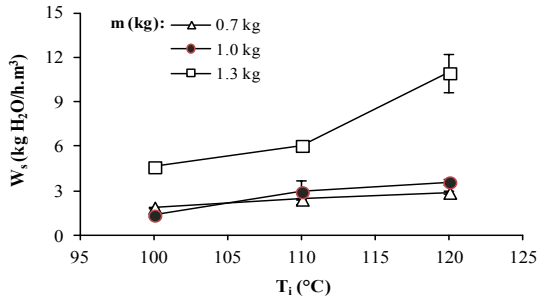


Figure 4. Relationship between specific water evaporation rate (W_s) and inlet temperature (T_i) for various mass of inert particles (m) when running at F = 1,5 kg/h and Q = 323 m^3/h.

3.3 Product Recovery

It was observed during experiment that the product recovery obtained from the drying of sweet corn milk in a bed of inert particles ranged between 2 to 89% depending upon the drying conditions. The drying process with product recovery of less than 60% might be classed as infeasible since it could not get sufficient amount of solid in the final product.

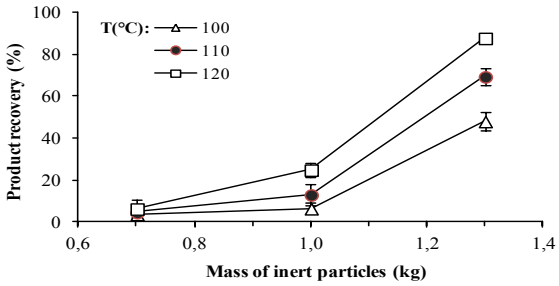


Figure 5. Relationship between product recovery (P_R) and mass of Teflon pellets (m) for various inlet air temperature (Ti) when running at F = 0,6 kg/h and Q = 323 m^3/h.

Table 1 shows that the mass of inert particles was the most significant factors affecting the product recovery with correlation coefficients of 0.718, followed by the feed rate (r = -0.289) and inlet air temperature (r = 0.286). In general, an increase in the mass of Teflon pellets seemed to result in increased levels of the product recovery. Typical plots of product recovery under selected experimental conditions of sweet corn milk drying are shown in Figures 5. When the drying of sweet corn milk in the experimental unit was carried out under steady-state operation, the product recovery reached maximum values close to 89 %. It is depicted in Figure 5 that the higher product recovery was obtained mostly when drying process was run using higher mass of inert particles and inlet air temperature. This was possibly due to the provision of heat and an adequate agitation of the Teflon pellets that could overcome the accumulation of agglomerated sweet corn milk inside a drying chamber so that can be recovered in the outlet section.

3.4 Drying Efficiency

The drying efficiency (η_d) of sweet corn milk in SVB of inert particles was approximately measured using Eq. 2. The present drying study on sweet corn milk showed the drying efficiency ranged between 6 and 48% depending upon drying conditions

It is shown in Table 1 that the drying efficiency obtained from this work were significantly correlated with the mass of inert particles and the inlet air temperature with correlation coefficients of about 0.650 and 0.416, respectively. Accordingly, the drying efficiency could be increased by adding the mass of.

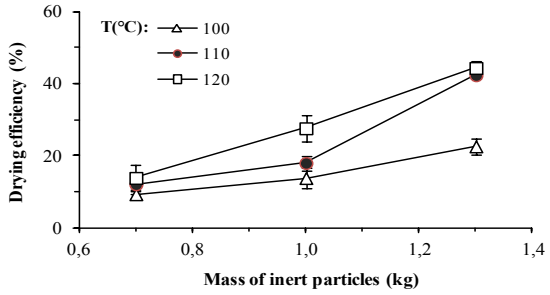


Figure 6. Relationship between drying efficiency and mass of inert particles (m) for a variety of the inlet air temperature (T_i) when running at F = 0,6 kg/h and Q = 323 m^3/h.

Teflon pellets and inlet air temperature during sweet corn milk drying in bed of inert particles. Figure 6 shows that an increase in amount of inert particles and inlet air temperature in a drying chamber increase the drying efficiency. Additional Teflon pellets improved its absorption capacity on existing heat inside a drying chamber that might decrease the outlet air temperature. This allowed more heat deposited in the inert particles that available for conductive heating of sweet corn milk during drying process. Consequently, it increased the sweet corn milk evaporation capacity by the dryer or in other words it increased drying efficiency.

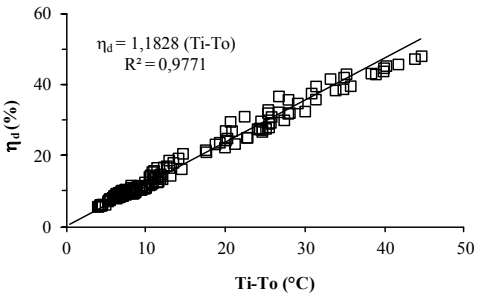


Figure 7. Linear relationship between drying efficiency (η_d) and temperature difference (T_i -T_o) obtained in the present study.

The drying efficiency increased linearly with an increase in the inlet-outlet air temperature difference as shown in Figure 7. It is confirmed that the drying efficiency may be increased by extending the gap between the air inlet and outlet temperatures, which can be realized by increasing the inlet air temperature or by dropping the outlet air temperature in several ways including the additional mass of inert particles.

4. Summary

Fresh sweet corn milk containing moisture of 91-95% (wb) could be dried successfully in a spouted-vortex-bed of inert particles into sweet corn milk powder with moisture content between 3 and 12 % (wb) depending upon the operating conditions. It appears that the mass of inert particles and the inlet air temperature were superior in affecting the SVB dryer performances such as the product moisture content, the specific water evaporation, the product recovery and the drying efficiency.

5. Acknowledgement

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Characteristics of Encapsulated Coffee Oil Produced by Variation of Gum Arabic and Coffee Oil Concentrations

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Abstract - The aim of the research was to know the characteristic of encapsulated coffee oil produced by variation of gum Arabic and coffee oil concentrations. Encapsulation was done using different concentration of gum Arabic (10 and 15% w/v) and concentration of coffee oil (10, 22.5 and 30% w/w toward gum Arabic) by spray drying method. Encapsulated coffee oil with 15% gum Arabic showed the higher encapsulation efficiency, higher value of hygroscopicity and lightness, stronger coffee aroma and lower oxidative stability than encapsulated coffee oil with 10% gum Arabic. As long as using the higher concentration of coffee oil, the encapsulation efficiency, hygroscopicity, lightness and oxidative stability were decreased, whereas coffee aroma was stronger. The best treatment was encapsulation using 15% of gum Arabic and 22.5% coffee oil which had encapsulation efficiency 50.04%, hygroscopicity 16.07g/100g, malonaldehyde 1.81 mmol/kg, lightness 75.26 and score of aroma 5.1 (coffee aroma was rather strong).

Keywords : Arabic gum, coffee oil, encapsulation

1. Introduction

Coffee oil can be obtained from green bean coffee (green coffee oil) or roasted been coffee (roasted coffee oil). The content of triglycerides in both oil are no significantly different [1]. Upon roasting, the Maillard reaction, Strecker degradation, pyrolysis, and other chemical reactions produce a large number of different volatiles. [2]. Coffee oil can be used to increase the aromatic potential of the soluble coffee and coffee beverages, as well as a flavoring for candies, cakes and puddings [3]. The main fatty acids found in coffee oil are linoleic and palmitic acid. Significant amounts of stearic and oleic acid are also present. [4]. Linoleic and oleic acids were unsaturated fatty acid which susceptible to oxidation. Oxidation can lead to the formation of unpleasant tastes and odors. Encapsulation has been proposed as an alternative to avoid such degradation processes.

Encapsulation consists of involving a solid, liquid or gaseous component in a wall material, in order to form a particle that may offer protection against oxygen, heat, humidity and light. It has been used by many researchers in order to promote better protection against lipid oxidation as well as better volatile retention, thus increasing the shelf life of oils and flavors [5,6,7,8].

The most common procedure for microencapsulation is spray drying. Many researchers have used the spray drying process to encapsulate oils and flavors such as cardamom oleoresin [9] and cinnamon oleoresin [10].

Due to its high encapsulation efficiency and good volatile retention, gum Arabic has been widely used as wall material in the microencapsulation of oils and flavors [8, 9,11]. It consists of a natural gum with good emulsifying properties, since it has a little protein content in its composition. Moreover, it exhibits high solubility and low viscosity in aqueous solution, which facilitates the spray drying process. The aim of the research was to know the characteristic of encapsulated coffee oil produced by variation of gum Arabic and coffee oil concentrations.

2. Materials and Methods

2.1 Materials

Coffee Arabica was obtained from coffee farmer at Bondowoso, East java, Indonesia. Gum Arabic, soya lechitin (Brataco), sodium chloride, TBA reagent, isobutanol, ethanol, hexane, (Merck), and distilled water were used in this research.

2.2 Coffee oil extraction

Coffee was roasted at 180^{O}C for up to 8 minutes, ground ad shifted with 60 mesh shift. Oil was extracted from coffee powder with hexane by soxhlet method, hexane was removed from coffee oil by rotary evaporator. Coffee oil was stored in dark bottle.

2.3 Coffee oil encapsulation

Wall solution was made by completely dissolved gum Arabic in warm distilled water, under magnetic stirring (G1 = 10% w/v and G2 = 15% w/v). Coffee oil was added to wall solution (C1 = 15% w/w, C2 = 22.5% w/w and C3 = 30% w/w to gum Arabic).

Lechitin (10% w/w to gum Arabic) was also added to wall solution to increase the emulsion stability. Emulsion of wall solution, coffee oil and lecithin was prepared using homogenizer Ultra Turax IKA T 150 operating at 5,200 rpm for 10 minutes.

Emulsions were spray dried in a laboratory scale spray dryer (Lab Plant Spray Dryer SD-05). The emulsion was fed by a peristaltic pump, at 0.3 l/h of feed flow rate, air flow rate at 36 m^3/h, compressed air flow rate at 2.4 m^3h, inlet temperature at 150°C and outlet temperature at 100°C. Encapsulated coffee oil was evaluated their characteristic, i.e. encapsulation efficiency, hygroscopicity, oxidative stability, lightness, and sensory test. Data was obtained from duplicate, presented as figure or table, and descriptive analysis was used in this research.

Encapsulation efficiency

Encapsulation efficiency (EE) was determined by the fraction of encapsulated oil over the total amount of oil (eq. 1)

EE = [(Oil Total-Oil Surface)/OilTotal] x 100 (1)

Where Oil Total is the total amount of oil and Oil Surface is the amount of non-encapsulated oil presented in the surface of microcapsules. The non-encapsulated oil present in the particles surface was determined according to reference [12]. Fifteen milliliters of hexane were added to two grams of powders in a sealed glass bottle, which was shaken for two minutes at room temperature in order to extract the free oil. Then the solvent mixture was passed through a number 1 Whatman paper filter. The powder collected in the filter was washed three times with 20 ml of hexane. The solvent was evaporated at 60 ºC until constant weight and the non-encapsulated oil was calculated based on the difference between the initial clean flask and that containing the extracted oil residue.

Hygroscopicity

Hygroscopicity was determined according to the method proposed [13], with some modifications. Samples of about one gram were placed in desiccators with a saturated solution of NaCl (relative humidity of 75.3%). After one week, samples were weighed and hygroscopicity was expressed as the amount of adsorbed moisture per 100 g of sample (g/100 g).

Oxidative stability

Oxidative stability of encapsulated coffee oil was measured after storing 3 month in dark condition (wrapped with alumunium foil) at ambient temperature. Oxidative stability was expressed as mmol malonaldehyde/kg which measured with TBA test [14]. Samples of about 0.05 gram was placed into test tube, 1 ml TBA reagent was added to the samples, then entered to boiled water bath for about 15 minutes. After being cooled 1 mL isobutanol and 5 mL ethanol were added, then being vortex. Malonaldehyde was measured spectrophotometrically at 481 nm. The concentration of malonaldehyde was expressed as mmol/kg malonaldehyde = [Absorbancy cm^{-1} (sample - blanko) x1000mM/Mml sample x 1000g/kg]/(1.56 x 10^5 M^{-1} x g sample x 100 mL/L).

Lightness

Lightness was measured with color reader (Minolta CR 300).

Sensory test

Sensory test was done by scoring method. Ten selected panelists were employed to evaluate the aroma of encapsulated coffee oil. 2 g of encapsulated coffee oil was placed into tight covered bottle. Panelists were instructed to open the bottle, bring it near the nose and sniff for 1-2 seconds. Sniffing between two samples was done at least after 5 seconds interval. Finally the panelists were instructed to score aroma of the sample, the scores were ranged from 1 = there was no aroma of coffee, till 7 = aroma coffee was very strong.

3. Results and Discussions

3.1 Encapsulation efficiency

The values of encapsulation efficiency are shown in figure 1. Encapsulation efficiency ranged from 40.20 – 54.09%. The highest value of encapsulation efficiency was obtained for encapsulated coffee oil produced with gum Arabic 15% and coffee oil 15%. Encapsulated coffee oil produced with gum Arabic 15% showed higher encapsulation efficiency than gum Arabic 10%. This result was accordance with the research of [15], which reported that increasing of wall material concentration could improve active substance retention and accelerated the formation of firm wall material which surrounded the droplet of active substance. Increasing coffee oil concentration reduced the encapsulation efficiency, because coffee oil did not perfect surrounded by wall material. This result was accordance with the research of [16, 17].

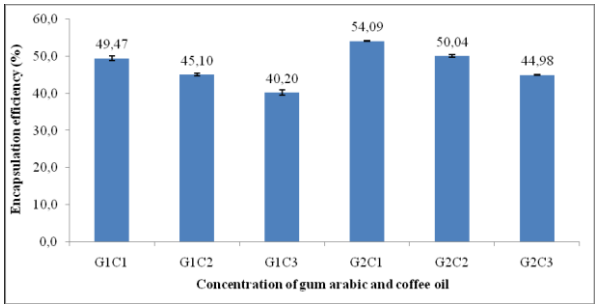

Figure 1: Encapsulation efficiency of encapsulated coffee oil

3.2 Hygroscopicity

Water adsorption is a critical factor in microencapsulated oils, since the presence of water can influence the lipid oxidation and powders flowability. Figure 2 shows the hygroscopicity of encapsulated coffee oil. The hygroscopicity ranged from 13.90 – 16.97. The highest value of hygroscopicity was obtained for encapsulated coffee oil produced with gum Arabic 15% and coffee oil 15%. Encapsulated coffee oil produced with gum Arabic 15% showed higher hygroscopicity than gum Arabic 10%. Gum Arabic presents a high number of ramifications with hydrophilic groups that easily bind to water molecules [18]. Increasing coffee oil concentration reduced the hygroscopicity, which could be expected, since coffee oil is a hydrophobic material, which does not adsorb water [3].

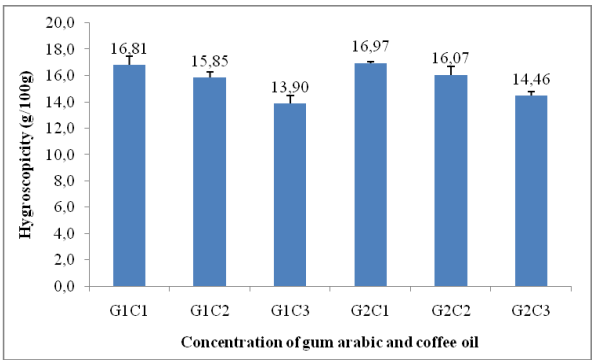

Figure 2: Hygroscopicity of encapsulated coffee oil

3.3 Oxidative stability

Malonaldehyde is one of the products of oxidation of oil, therefore malonaldehyde can be used as an indicator of oxidative stability. Increasing of malonaldehyde indicated the lower oxidative stability. The concentration of malonaldehyde of encapsulated coffee oil after storing 3 months at ambient temperature is shown in figure 3. Malonaldehyde concentration ranged from 1.52 – 1.98 mmol/kg. The highest value of concentration of malonaldehyde which is indicated the lowest oxidative stability was obtained for encapsulated coffee oil produced with gum Arabic 15% and coffee oil 30%. Encapsulated coffee oil produced with gum Arabic 15% showed lower oxidative stability than gum Arabic 10%. Encapsulated coffee oil produced with gum Arabic 15 % showed higher higroscopicity, it could accelerate lipid oxidation, therefore reduced the oxidative stability. Increasing coffee oil concentration reduced the oxidative stability, because coffee oil contain unsaturated fatty acids which susceptible to oxidation [4].

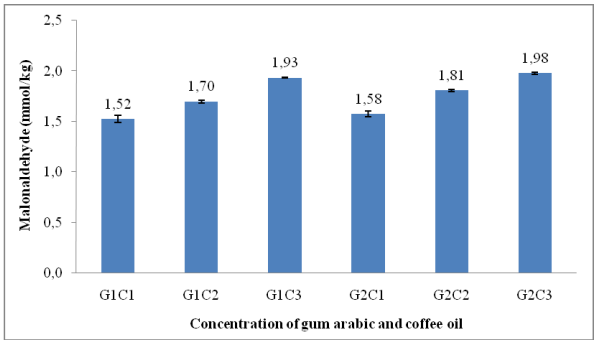

Figure 2: Malonaldehyde concentration of encapsulated coffee oil

Molonaldehyde concentration of fresh coffee oil was 1.58 mmol/kg, and after storing 3 months in the dark bottle at ambient temperature became 268,2 mmol/kg. The highest concentration malonaldehyde of encapsulated coffee oil was 1.98 mmol/kg capsul. If converted to kg oil it became 19,22 mmol/kg oil. This data indicated that encapsulation was effective to retard the oxidation of coffee oil.

3.4 Lightness

The values of lightness of encapsulated coffee oil are shown in Table 1. The values of lightness ranged from 74.17 – 75.64. The highest value of lightness was obtained for encapsulated coffee oil produced with gum Arabic 15% and coffee oil 15%. The color of gum Arabic is white, whereas coffee oil is brown. Therefore encapsulated coffee oil produced with gum Arabic 15% showed higher lightness than gum Arabic 10%, and increasing coffee oil concentration reduced the value of lightness.

Table 1. Lightness of encapsulated coffee oil

Treatment	Lightness
G1C1	75,17 ± 0,36
G1C2	75,00 ± 0,09
G1C3	74,17 ± 0,09
G2C1	75,64 ± 0,18
G2C2	75,26 ± 0,18
G2C3	75,13 ± 0,36

3.5 Sensory test

Sensory test for aroma encapsulated coffee oil are shown in figure 4. Score of aroma encapsulated coffee oil ranged 4.2-5.3 (aroma coffee was rather strong). Encapsulated coffee oil produced with gum Arabic 15% showed a slight higher score of aroma than gum Arabic 10%. Increasing coffee oil concentration increased score of aroma encapsulated coffee oil, which could be expected, since coffee oil was source of aroma which generated from roasting processing.

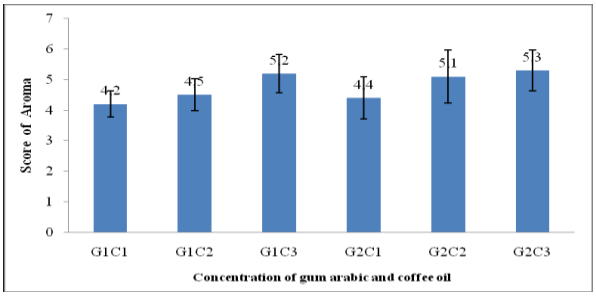

Figure 4. Score of aroma encapsulated coffee oil

4. Conclusions

Encapsulated coffee oil with 15% gum Arabic showed the higher encapsulation efficiency, higher value of hygroscopicity and lightness, stronger coffee aroma and lower oxidative stability than encapsulated coffee oil with 10% gum Arabic. As long as using the higher concentration of coffee oil, the encapsulation efficiency, hygroscopicity, lightness and oxidative stability were decreased, whereas coffee aroma was stronger. The best treatment was encapsulation using 15% of gum Arabic and 22.5% coffee oil which had encapsulation efficiency 50.04%, hygroscopicity 16.07g/100g, malonaldehyde 1.81 mmol/kg, lightness 75.26 and score of aroma 5.1 (coffee aroma was rather strong).

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PP eco-friendly Extraction of Banana Agro-industry Waste and its Functional Groups

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Abstract **-** Pectin or pectic polysaccharides is a naturally-occurring polysacharride found in many plants. Its effects on health is receiving growing interest for their applications such as prebiotic ingredient. In this work, pectin was isolated from banana agro-industry waste (peel and bunches of bananas) by using water extraction method. There were three methods i.e. one time, two times and three times of extraction. The extracted pectin was characterized by FTIR. The result showed that pectin extraction method was twice (two times phase of extraction) as much as the extraction stage effective and efficient to extract the pectin than one or three times extraction. The results of functional groups analysis was using FTIR instrument indicated that the peak of extracted pectin had the same functional group with standard pectin. But there were impurities (ethanol compound) as a effect of the analysis performed on wet samples (pectin has not dried). Functional groups presented in the pectin from banana waste i.e intermolecular alcohol group, a carboxylic acid dimer, free carbonyl, amine compounds and group of primary and secondary aliphatic.

Keywords**:** eco-friendly extraction, pectin/pectic polysaccharides, FTIR.

1. Introduction

Pectin is a naturally-occurring polysacharride found in many plants. Its effects on health is receiving growing interest for their applications such as prebiotic ingredient. Peel and banches of banana contain pectin up to 4% of the dry weight in the peels [1], and 10% of the dry weight in the bunches [2].

The properties of pectin are soluble in water especially in the hot water [3]. Pectin is used to improve the viscosity, stability, texture, and appearance of food products [4]. Pectin is also used to gel formation and a stabilizer in fruit juices, jelly-making materials, jem and marmalade [5].

Low methoxyl pectins (< 50% esterified) form thermoreversible gels in the presence of calcium ions and at low pH (3–4.5) whereas high methoxyl pectins rapidly form thermally irreversible gels in the presence of sufficient (for example, 65% by weight) sugars such as sucrose and at low pH (< 3.5); the lower the methoxyl content, the slower the set [6].

Extraction of pectin usually done by heating the material at a given temperature in a acid solution such as HCl [7]. Hydrochloric acid solution is less environmentally friendly when used as a solvent to pectin extraction. Hutagalung [1] and Laksono [2] has been extracting pectin from banana peel and bunches at 60^{o}C. While Tuhuloula [8] conducted the pectin of kepok var. banana peel 80^{o}C. Extraction of pectin can be done by extracting a single stage and multi-stage [9]. Extraction of one stage is the extraction with a suitable solvent at the same amount, so it takes a lot of solvent to dissolve the pectin. Ekstraksi is a multi-step solvent extraction with the addition of the new always on the residue from the previous extraction of pectin dapatterekstrak so optimally [10]. Therefore the research used water as the eco friendly solvent to extract the banana pectin with combined temperature (60 ^{o}C and 80^{o}C) and phase extraction (one time, two times and three times).

2. Materials and Methods

2.1 Plant Material

Banana peel and bunches were obtained as a waste material from agroindustry (banana home industry) produced banana unripe chips. The materials were collected from plantain (agung var.) and banana (embug var.). The materials were cut into pieces and dried under shade for 24-48 h, further dried at 30–40ºC until constant weight was obtained. Dried fruit peel was powdered in to electric grater. Powdered peel was further passed from sieve 60 and stored in air tight container until used.

2.2 Eco-friendly Extraction of Pectic polisaccharides (PP)

Pectins were extracted from the cell-wall material (CWM) by sequential extraction with water (1 × 1h; 2 × 1h; 3 × 1 h at 60 ^{o}C) according to the method

established by Emega [11]. All extracts were recovered by filtration and precipitated with four ethanol volumes, providing a water-soluble pectin (WSP) as shown in the fraction scheme (Fig. 1).

2.3 Analysis of Moisture Content of The Banana Peel-Bunches Powder and Yield of Pectin

Moisture content of banana peels and bunches powder were determined by ovendrying [12], using an air-circulated oven at 106 ^{o}C, for 24 h. All values were calculated on a dry-weight basis. Pectins were extracted from the banana peels and bunches by sequential extraction with water. After each extraction step, the insoluble residues were not dried to avoid further irreversible collapse of cell-walls, which can hinder the following extractions. The final residue was dried and weighed. The resulting polysaccharides in the extracts are named water-soluble pectin (WSP). The yield of pectin was calculated on a wet-weight basis based on this formula

Yield (%) = [weight of wet pectin/weight of the powder] × 100.

2.4 Analysis of PP functional groups by the fourier transform IR spectrum (FT-IR)

The Fourier transform IR spectrum (FT-IR) of pectin was recorded using a Bruker Optics ALPHA IR spectrophotometer. The FT-IR was operated in the range of 500 and 4000 cm^{-1} as KBr pellet [13].

One time phase : WSP 1
Two times phase : WSP 1 + WSP 2
Three times phase : WSP 1 + WSP 2 + WSP 3

Fig. 1. Scheme of extraction of pectic polysaccharides from banana peels and bunches

3. Result and Discussion

3.1. Moisture content of banana peels and bunches powder

The moisture content varied based on their characteristic. Characteristic of banana peels was different with banana bunches. The moisture content of the waste banana powder ranged from 8.14 to 9.05% (Figure 2).

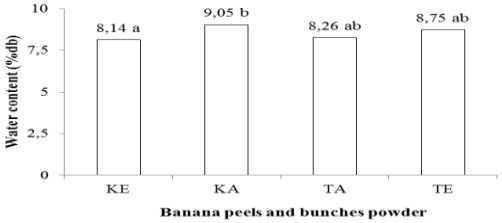

Fig 2. Moisture content of banana peels and bunches powder: agung var peel (KA), embug var peel (KE), agung var bunches (TA), embug var bunches (TE)

Agung var banana peel powder had a higher moisture content (9.05%) was compared with embug var. banana peel (8.14%). The moisture content difference on the banana peel was significantly different after further tested with LSD at the level of $\alpha \leq 5\%$. Embug var. bunches powder had a higher water content (8.75%) than the great banana bunches (8.26%), but was not significantly different at a further test LSD at $\alpha \leq 5\%$ level.

3.2 Effect of varieties and material kind to yield of banana pectin

For peels, the variation in both varieties (agung var. and embug var.) were significant (LSD test) for pectin content (Figure 3). The pectin content of embug var. peel (4.54%) was higher than agung var. peel (4.38%) and showed significantly different after further tested with LSD at the level of $\alpha \leq 5\%$. The pectin content of embug var. bunches (2.76) was higher than the great banana bunches (2.68%) but no significantly different.

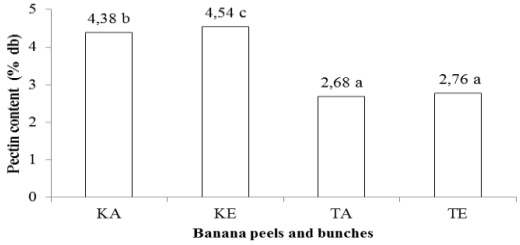

Fig 3. Pectin content of banana peels and bunches powder: agung var peel (KA), embug var peel (KE), agung var bunches (TA), embug var bunches (TE)

The kind of banana also affect pectin content. The banana kind have a higher pectin content than the plantain kind. Tuhuloula [8] reported that the pectin content of ambon var. (banana) was higher than kepok var. (plantain). Physical characteristic showed that ambon var. was more bigger than kepok var., so be expected more protopectin can be hydrolyzed into pectin.

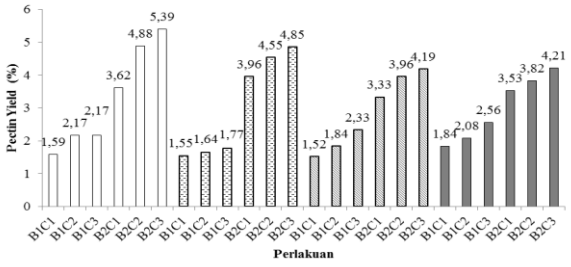

Fig 4. Pectin yield of embug var peel ( ), agung var peel), embug var bunches ( ), agung var bunches) at extraction temperature 60^{o}C (B1), 80^{o}C (B2) for one time phase (C1), two times phase C2) and three times phase (C3).

Figure 4 showed that the pectin extraction was conducted at 80 ^{o}C resulted the greater of pectin yield. These was due to high extraction temperatures help the diffusion of the solvent into the network and increase the activity of the solvent in the pectin hydrolyzing [14]. The increase in kinetic energy resulting in the release of polysaccharides from tissue cells that yield more (Nurdjanah and Usmiati, 2006).

Bernasconi [9] reported that the one phase of extraction method was generally not possible to soluble because was due to the equilibrium between the soluble extracts and the extracts. Extraction would be more beneficial if was done in a many number of stages and each stage uses little solvent. The yield of pectin was obtained with the optimal extraction method two times phase.

3.3 Infrared spectroscopy

IR spectroscopic study revealed the presence of characteristics group in the extracted pectin. Peak of carboxylic acid group easily identified in the banana peel and bunches derived pectin. An overview of the IR spectrum of pectin is shown in Figure 5 the "fingerprint" region of the spectrum (up to approx. 2000 cm^{-1}) includes the region of 1200-1800 cm^{-1} as shown.

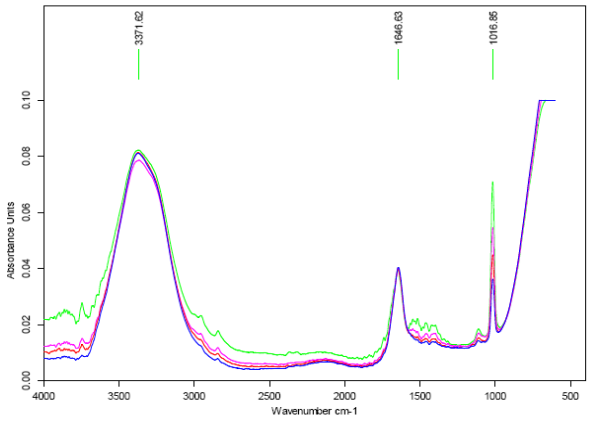

Fig 5. FTIR spectrum of pectin from banana peels and bunches: embug var. peel (--), agung var. peel (--), embug var. bunches (--), agung var. bunches (--)

The result can observed the region that characterizes the state of carboxylic groups (approx. 1750-1350 cm^{-1}). The band at approx. 1743 cm^{-1} was indicative of the stretching group C = O of non-ionized carboxylic acid (methylated or protonated). Its ionization (formation of salt) leads to their disappearance, and the appearance of stretch modes of COO^{-} in approximate 1600-1650 and 1400-1450cm^{-1}, respectively. The degree of methylation (DM) is defined as the amount of ester groups compared to the total amount of acid groups and carboxylic ester and it is observed that the high intensity of the band at 1743 cm^{-1} shows that the pectin obtained is of high degree of methylation.

4. Conclussion

Banana peel contain more pectin than banana bunches at agung and embug var. Two times phase of pectin extraction was more effective and efficient than one time or three times phase. The extracted pectin contain impurities (ethanol compound) as a effect of the analysis performed on wet samples (pectin has not dried). Functional groups presented in the pectin from banana waste i.e intermolecular alcohol group, a carboxylic acid dimer, free carbonyl, amine compounds and group of primary and secondary aliphatic. Further research can be conducted about the rheological study of continuous shear of pectin solution.

5. Acknowledgments

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Application of Fuzzy Time Series in Forecasting of Property Prices in Malaysian Stock Market

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Abstract - Fuzzy Time Series is the one of the methods that has been used for forecasting problems. Forecasting is important for the organizations to control their activities. Fuzzy Time Series method can be used to know the trend of development of their organizations. In this paper we will apply Fuzzy Time Series Technique to forecast the property prices in Malaysia using data from Malaysia Share Market from year 1992 until 2013.

Keywords: Fuzzy Time Series, Share Market Index, forecasting, historical data.

1. Introduction

Fuzzy Time Series is the one of the method that has been used for forecasting problems. Forecasting is important for the organizations to control their activities. Fuzzy Time Series method can be used to know the trend of development of their organizations.

The data that was collected by researchers before were in linguistic terms, and also the statistical methods that were used were not appropriate. In order to cope with this problem, Fuzzy Time Series method has been proposed in forecasting the property price in stock market. In [6] a proposal of Fuzzy Time Series method involving these problems.

2. Literature Review

1.1. Introduction

Fuzzy Time Series is one method that can be used in forecasting the stock market prices [8]. Forecasting performance is affected by fuzzy relation matrix which is derived by an arithmetic procedure. Li and Xiong [5] introduced one method to forecast financial time series which is Fuzzy Neural Networks. That method is a class of adaptive networks and same function as fuzzy inference system.

The forecasting of stock market prices is basically to see how the patterns of the graphs for stock market prices. Chen et al.[1] proposed Fuzzy Time Series based on rough set to see how the patterns of fluctuations can be extracted. There are other researchers that used Fuzzy Time Sequence in stock prediction. The stock market prices was forecasted reasonable by using Fuzzy Time Series [3].

In order to cope with the complexity of stock price, Jin et al. [4] introduced a hybrid method, autoregressive and moving average (ARMA), back propagation neural network (BPNN) and Markov model in stock prediction. Most of the researcher's proposing various methods in stock prediction is basically with the aim to improve the accuracy of that particular predictions.

2.2. Stock Market Prices

Stock market is one of the famous environments among the investors in buying or selling shares. It is often taken as the primary indicator in economy of a particular country. An economy is considered to be upcoming economy when the stock market prices are high. So, it is important to know the patterns of stock market prices. Schneider and Shuhadah [7] used fuzzy logic in decision making for stock trading in order to assist investors in making decision on their shares.

2.3 Fuzzy Time Series Analysis

We would like to see the patterns of the graphs of proposed method compared with standard method. Good prediction accuracy for stock price in short term is discussed in Ghanbari et al. [3] by extracting useful patterns of information with a descriptive rule induction approach. There are four types of patterns for these time series which are secular trend, seasonal variation, cyclical variation, and irregular variation [8].

3. Methodology

We use the data obtained from Kuala Lumpur Stock Exchange (KLSE) as shown in yahoo finance website. There are 8 steps that must be considered. Figure 1 shows those steps.

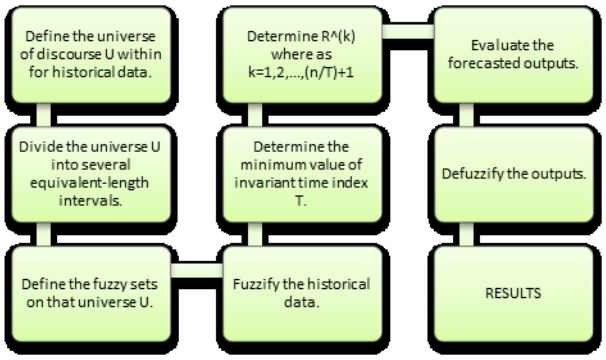

Figure 1: Steps in Fuzzy Time Series method.

Step 1, the universe of discourse must first be determined.

Step 2, From the historical data, we find the D_{max} and D_{min}. The property prices in the stock market price historical data will be divided to n equal-length intervals denoted by $u_1, u_2, \ldots u_n$ where the values of the interval depending on values of D_{max} and D_{min}. So, the universe is the interval of $U = [D_{min} - D_1, D_{max} - D_2]$ where D_1 and D_2 are two positive numbers.

In step 3 and 4, fuzzy sets on particular universal discourse U is defined where $\tilde{A}_i (i = 1,2, \ldots, m)$.

$$\tilde{A}_i = \{(\mu_{\tilde{A}_i}(u_j)/u_j) | \mu_{\tilde{A}_i}(u_j) \in [0,1], u_j \in R \; j = 1,2, \ldots, m\}$$

with the membership degree $\mu_{\tilde{A}_i}(u_j)$ of u_j. Then, define $\tilde{A}_1, \ldots, \tilde{A}_m$. After that, proceed to find an equivalent fuzzy set for each input data where we need to define a cut set for each $\tilde{A}_i (i = 1,2, \ldots, m)$.

Step 5, determine fuzzy relation matrix R. Let a fuzzy relation matrix R be an m x m matrix, then the nth order fuzzy relation matrix R^n is shown as follow.

$$R^n = R^{n-1} \circ R$$

Where $\circ$ is the 'max-min' operator. $d(R^{T+1}) = d(R^T), T \in [1, n]$ at steady state. Thus, by assuming $K = 1$, the formula can be derived as below to determine the minimum T.

$$\min T$$
$$subject\ to\ r_{ij}^{T+1} = r_{ij}^{T} \quad \forall_{i,j} = 1,2, \ldots, m$$
$$0 \leq r \leq 1.$$

The objective value T is obtained to get the values of $[n/T] + 1$ time invariant relations matrices per T time-series data. After that, by using fuzzy relation matrix $R^k \; \forall k = 1,2, \ldots, [n/T] + 1$, the forecasting output $F(t)$ could be obtained by input fuzzy data $\tilde{A}_i (i = 1,2, \ldots, m)$ as follow.

$$F(t) = \tilde{A}_i \circ R^k, \qquad \forall (t-1) \leq k \cdot T, \qquad k = 1$$

Lastly step 6, fuzzy output is needed to defuzzy into real numbers since the values of forecasting output is all in fuzzy sets. There are suppose to have three criteria in defuzzify the output [2].

1) Select the midpoint of the interval corresponding to the maximum as the forecasted value if the membership of an output has only one maximum.
2) Select the midpoint of the corresponding conjunct intervals as the forecasted value if the membership of an output has one or more consecutive maximums.
3) Otherwise, standardize the fuzzy output and use the midpoint of each interval to evaluate the centroid of the fuzzy set as the forecasted value.

Example 2.1 [8]

Using the above example, the whole steps will be shown in details. In this section the historical data of property prices for year 1992 till 2013 will be used to compare the result with the forecasting values using fuzzy time series.

Step 1: Here, the universal discourse is property prices. The diagram below shows the historical data property prices by year 1992 till 2013.

Table 3.1. Historical data of property prices in Malaysia for year 1992 till 2013.

Year	Historical data	Year	Historical data
1992	1139.79	2003	750.7
1993	3370.43	2004	716.98
1994	2559.99	2005	524.64
1995	2103.72	2006	693.49
1996	2538.8	2007	1035.66
1997	760.9	2008	515.61
1998	781.42	2009	781.71
1999	1026.68	2010	1020.86
2000	632.54	2011	999.71
2001	599.64	2012	1053.71
2002	540.91	2013	1293.35

Step 2: From the historical data, find D_{max} and D_{min}, where D_{max}= 3370.43 and D_{min}= 515.61. Proceed by divide historical data into 6 equal-length intervals denoted by u_1, u_2, u_3, u_4, u_5, and u_6. The values of the interval depending on values of D_{max} and D_{min}. So, the universe in this case, U = [500, 3500] where as D_1 = 15.61 and D_2 = 629.57. The intervals that we get are

$u_1 = [500,1000]$, $u_2 = [1000,1500]$, $u_3 = [1500,2000]$,
$u_4 = [2000,2500]$, $u_5 = [2500,3000]$, and $u_6 = [3000,3500]$.

Step 3: Here, the historical data of property prices can best be described by the fuzzy sets $\tilde{A}_1$=very low. $\tilde{A}_2$=not too low. $\tilde{A}_3$=low. $\tilde{A}_4$=high. $\tilde{A}_5$=very high. $\tilde{A}_6$=too high. Each fuzzy set $\tilde{A}_i$(I = 1,2,…6) is defined as

$$\tilde{A}_i = \{(\mu_{\tilde{A}_i}(u_j)/u_j) | \mu_i(u_j) \in [0,1], u_j \in R \; j = 1,2, \ldots 6\}$$

with the membership degree $\mu_{\tilde{A}_i}(u_j)$ of u_j as follow.

Table 3.2. Membership degree of historical data of property prices.

Fuzzy Sets/Intervals	u_1	u_2	u_3	u_4	u_5	u_6
A_1	1	0.5	0	0	0	0
A_2	0.5	1	0.5	0	0	0
A_3	0	0.5	1	0.5	0	0
A_4	0	0	0.5	1	0.5	0
A_5	0	0		0.5	1	0.5
A_6	1	0.5	0	0	0.5	1

Step 4: Fuzzify the historical data in order to determine an equivalent fuzzy set for each input data. In other words, fuzzification is the process of making crisp quantity of fuzzy sets. Each historical data value is fuzzified based on its highest degree of membership. According to table 3.1, it shows that the historical data in 1992 was 1139.79 which lies within the boundaries of interval u_1 and since its highest membership degree of u_1 occurs at $\tilde{A}_1$, thus the historical time variable $F(1992)$ is fuzzified as $\tilde{A}_1$. Actual historical data for property prices in 1993 is 3370.43 which lies within the boundaries of interval u_2. Thus $F(1993)$ is fuzzified as $\tilde{A}_1$. A complete overview of fuzzified historical data is shown in the table 3.3.

Table 3.3. Fuzzified historical data

Year	Historical data	Interval	Fuzzified historical data
1992	1139.79	[1000, 1500]	A_2
1993	3370.43	[3000, 3500]	A_6
1994	2559.99	[2500, 3000]	A_5
1995	2103.72	[2000, 2500]	A_4
1996	2538.8	[2500, 3000]	A_5
1997	760.9	[500, 1000]	A_1
1998	781.42	[500, 1000]	A_1
1999	1026.68	[1000, 1500]	A_2
2000	632.54	[500, 1000]	A_1
2001	599.64	[500, 1000]	A_1
2002	540.91	[500, 1000]	A_1
2003	750.7	[500, 1000]	A_1
2004	716.98	[500, 1000]	A_1
2005	524.64	[500, 1000]	A_1
2006	693.49	[500, 1000]	A_1
2007	1035.66	[1000, 1500]	A_2
2008	515.61	[500, 1000]	A_1
2009	781.71	[500, 1000]	A_1
2010	1020.86	[1000, 1500]	A_2
2011	999.71	[500, 1000]	A_1
2012	1053.71	[1000, 1500]	A_2
2013	1293.35	[1000, 1500]	A_2

Step 5: In this section, determine fuzzy relation matrix R. Based on the Table 3.3, the fuzzy relation matrix is defined as $\tilde{A}_2 \rightarrow \tilde{A}_6$, $\tilde{A}_6 \rightarrow \tilde{A}_5$, $\tilde{A}_5 \rightarrow \tilde{A}_4$, $\tilde{A}_4 \rightarrow \tilde{A}_5$, $\tilde{A}_5 \rightarrow \tilde{A}_1$, $\tilde{A}_1 \rightarrow \tilde{A}_1$, $\tilde{A}_1 \rightarrow \tilde{A}_2$, $\tilde{A}_2 \rightarrow \tilde{A}_1$, $\tilde{A}_2 \rightarrow \tilde{A}_2$

After define the fuzzy relation matrix, fuzzy relationship groups is established in the table 3.4.

Table 3.4. Fuzzy set relationship.

Group 1:	$\tilde{A}_1 \rightarrow \tilde{A}_1$	$\tilde{A}_1 \rightarrow \tilde{A}_2$	
Group 2:	$\tilde{A}_2 \rightarrow \tilde{A}_6$	$\tilde{A}_2 \rightarrow \tilde{A}_1$	$\tilde{A}_2 \rightarrow \tilde{A}_2$
Group 3:	$\tilde{A}_4 \rightarrow \tilde{A}_5$		
Group 4:	$\tilde{A}_5 \rightarrow \tilde{A}_4$	$\tilde{A}_5 \rightarrow \tilde{A}_1$	
Group 5:	$\tilde{A}_6 \rightarrow \tilde{A}_5$		

Step 6: Defuzzify the forecasted output of the fuzzified historical data of property prices of *F(t-1)* is $\tilde{A}_1$, thus forecasted output is defined according to the following principles:

1. If there subsists a one-to-one relationship in the relationship group of $\tilde{A}_j$, verbally express $\tilde{A}_j \rightarrow \tilde{A}_k$, and the highest degree of belongingness of $\tilde{A}_k$ occurs at interval u_k, then the forecasted output of *F(t)* is equals the midpoint of u_k.
2. If $\tilde{A}_j$ is empty, i.e. $\tilde{A}_j \rightarrow \emptyset$, and the interval where $\tilde{A}_j$ has the highest degree of belongingness is u_j, then the forecasted output equals the midpoint of u_j.
3. If there exists a one-to-many relationship in the relationship group of $\tilde{A}_j$, let say $\tilde{A}_j \rightarrow \tilde{A}_1, \tilde{A}_2, \ldots, \tilde{A}_n$, and the highest degree of belongingness occurs at $u_1, u_2, \ldots, u_n$, then the forecasted output is calculated as the average of the midpoints $m_1, m_2, \ldots, m_n$, of $u_1, u_2, \ldots, u_n$. This equation can be simplified as:

$$\frac{m_1 + m_2 + \cdots + m_n}{n}$$

4. Analysis and Result

Considering the above data from the historical data we get forcasted value as shown in Table 3.5.

The forecasting errors of property prices is shown in Table 3.6

Table 3.5. The forecasted values

Year	Actual value	Forecasted value	Fuzzy relationship groups	Interval midpoints
1992	1139.79		$A_2 \rightarrow A_6, A_1, A_2$	3250; 750; 1250
1993	3370.43	1750	$A_6 \rightarrow A_5$	2750
1994	2559.99	2750	$A_5 \rightarrow A_4, A_1$	2250; 750
1995	2103.72	1500	$A_4 \rightarrow A_5$	2750
1996	2538.8	2750	$A_5 \rightarrow A_4, A_1$	2250; 750

1997	760.9	1500	$A_1 \rightarrow A_1, A_2$	750; 1250
1998	781.42	1000	$A_1 \rightarrow A_1, A_2$	750; 1250
1999	1026.68	1000	$A_2 \rightarrow A_8, A_1, A_2$	3250; 750; 1250
2000	632.54	1750	$A_1 \rightarrow A_1, A_2$	750; 1250
2001	599.64	1000	$A_1 \rightarrow A_1, A_2$	750; 1250
2002	540.91	1000	$A_1 \rightarrow A_1, A_2$	750; 1250
2003	750.7	1000	$A_1 \rightarrow A_1, A_2$	750; 1250
2004	716.98	1000	$A_1 \rightarrow A_1, A_2$	750; 1250
2005	524.64	1000	$A_1 \rightarrow A_1, A_2$	750; 1250
2006	693.49	1000	$A_1 \rightarrow A_1, A_2$	750; 1250
2007	1035.66	1000	$A_2 \rightarrow A_8, A_1, A_2$	3250; 750; 1250
2008	515.61	1750	$A_1 \rightarrow A_1, A_2$	750; 1250
2009	781.71	1000	$A_1 \rightarrow A_1, A_2$	750; 1250
2010	1020.86	1000	$A_2 \rightarrow A_8, A_1, A_2$	3250; 750; 1250
2011	999.71	1750	$A_1 \rightarrow A_1, A_2$	750; 1250
2012	1053.71	1000	$A_2 \rightarrow A_8, A_1, A_2$	3250; 750; 1250
2013	1293.35	1750	$A_2 \rightarrow A_8, A_1, A_2$	3250; 750; 1250

Using the eight steps as shown, the result for the forecasting property prices is defined and shown in the table 3.6. After getting the forecasted value, the error is evaluated using the formula (1) to get an estimated error between the forecast value and the actual value.

$$\text{Forecast error} = \frac{|(\text{forecasted} - \text{actual})) \text{ value}|}{(\text{actual value})} * 100 \quad \ldots\ldots\ldots\ldots (1)$$

$$\text{Average forecasting error} = \frac{(\text{sum of forecasting errors})}{(\text{total number of errors})} \quad \ldots\ldots\ldots (2)$$

Table 3.6. the forecasting errors of property prices.

Year	Actual value	Forecasted value	Forecasting error (%)
1993	3370.43	1750	48
1994	2559.99	2750	7
1995	2103.72	1500	29
1996	2538.8	2750	8
1997	760.9	1500	97
1998	781.42	1000	28
1999	1026.68	1000	26
2000	632.54	1750	177
2001	599.64	1000	67
2002	540.91	1000	85
2003	750.7	1000	33
2004	716.98	1000	39
2005	524.64	1000	90
2006	693.49	1000	44
2007	1035.66	1000	3
2008	515.61	1750	239
2009	781.71	1000	28
2010	1020.86	1000	2
2011	999.71	1750	75
2012	1053.71	1000	5
2013	1293.35	1750	35

From the above actual and forecasted values we can plot the values on the same graph and see the pattern of the graph.

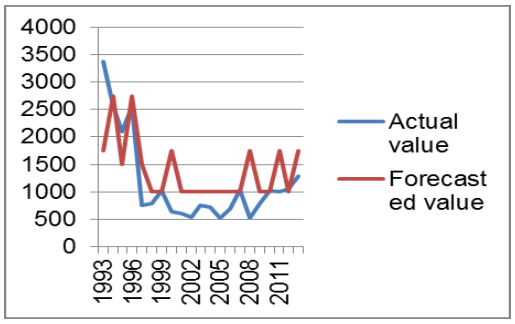

Figure 2. Forecasting observation of property prices by year 1993 till 2013.

From table 4.6, it shows that all forecasted values are close to the actual values. Definitely, it confirms that the model gives good predicting. The small value of the average forecasting error, thus it confirms the goodness of forecasting model. Based on figure 4.7, it can be seen that the pattern of the graph is an irregular variation of fuzzy time series.

5. Summary and Conclusion

Generally, Fuzzy time series method is good for predicting and forecasting problems in fluctuating series especially for stock market prices based on average lengths of interval. We use this method to forecast the stock market prices (property prices) and observe the pattern of the graph by using historical data. The linguistic values that have been used in this method gives us better decision and accuracy as compared to traditional methods.

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Neural Network-Based Prediction of Ionospheric Total electron Content (TEC) Using Various Training Algorithms

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Abstract - The space research community uses Neural Networks (NNs) as part of a tool to predict and model the complex and non-linear characteristics of the ionosphere. Eight different training algorithms (gda, rp, lm, bfg, cgb, cgp, scg, and cgf) within the NN toolbox of MATLAB were used to train the NN. This was done to assess the performance of the aforementioned algorithms in estimating the ionospheric total electron content (TEC). This paper presents a comparative study of the performance accuracy of each training algorithm with regard to ability to predict. The computational time (t) of the training and the correlation coefficient (R^2) of the linear regression line between the predicted value (NN_{TEC}) and the observed value (GPS_{TEC}) were monitored and used as performance criteria to determine the best training algorithm in estimation of TEC. The performance of the model showed that the LM (Levenberg- Marquardt) training algorithm achieved the fastest convergence rate during the training process. Furthermore, the LM algorithm achieved R^2 value greater than 0.9 in predicting TEC which is considered a well trained model and has high goodness of fit. Thus, the results of this study indicate that the appropriate selection of training algorithm is essential for the optimisation of NN models.

Keywords*:* Neural Network, training algorithm, total electron content

1. Introduction

Neural Networks (NNs) are well known empirical model used to forecast non linear and complex processes in various applications. The techniques were employed in space research studies and found to be promising in modelling the non linear behaviour of the ionospheric total electron content [1-6]. The total electron content (TEC) is a significant parameter that able to describe the behaviour and characteristic of ionosphere temporally and spatially.

A few features in NN have significant effect on the predictability of the resultant model such as the architectures of the network, training algorithms and activation function assigned in each neuron. Less attention has been paid to the effect of training algorithm on the predictive ability of the designed model in ionospheric research. Koker et al. [7] and Habarulema and McKinnell [8] have stated that proper selection of training algorithm able to minimize the error in the prediction model and maximize the predicting ability.

The main purpose of the current study is to investigate the performance of different training algorithms employed in TEC models with proper input parameters that have effect on the TEC variations. This study undertakes investigation of eight different training algorithms (GDA, RP, LM, BFG, CGB, CGP, SCG, CGF) using Neural Network toolbox of MATLAB. In this study NNs are used to predict the nonlinear TEC variability at a single station at Parit Raja (1.86°N, 103.8°E), Malaysia for the year 2005.

2. Techniques

NNs are information processing paradigm with high degree of interconnection processing element known as neurons. The arrangement of the neurons in each layers and the interconnection links within and between the layers are the key factors that distinguish different types of architectures in NNs. There are two types of network architectures, single layered or multilayered networks. A single layered is the simplest form of network which only has an input layer and an output layer whereas the multilayered networks consist of extra layers known as hidden layers located in between input and output layer.

In the present work, a multilayered feed forward (FFD) neural network is used with three layers, namely input, output and a single hidden layer. One of the well known learning algorithms called back propagation is used. The FFD back propagation is popular among the other algorithms due to its effectiveness in various applications and simplicity in developing a model for a complex environment [10]. On the other hand, a few researchers concluded that back propagation algorithm has its greatest contribution in non linear application and it is a universal non linear approximation [11, 9]. The NNs model capable to generalize once the model is well trained with sufficient and well-defined information.

2.1 Input Space

Prominent input output pairs would ensure the success of the designing process whereas improper pairs might cause the system fails to converge to an acceptable solution. Therefore an accurate and proper data in training could generalize well and will be able to develop a reliable model for the TEC forecasting purposes. Since Parit Raja station (1.86°N, 103.8°E) lies in the equatorial anomaly, the region is more subjected to large temporal and spatial variability.

The diurnal and seasonal variations of ionosphere are the dominant and significant variables at this region; therefore hour (HR) and the day number (DN) were included as the input parameters. The parameters were split into sine and cosine components to allow the data continuity [12] as below:

$$DNs = sin\left(\frac{2\pi * DN}{365.25}\right), \quad DNc = cos\left(\frac{2\pi * DN}{365.25}\right) \quad (1)$$

$$HRs = sin\left(\frac{2\pi * HR}{24}\right), \quad HRc = cos\left(\frac{2\pi * HR}{24}\right) \quad (2)$$

Furthermore the TEC variability is mainly influenced by solar and magnetic activity. The sunspot number (SSN) index will represent the solar activity because large variation in ionospheric TEC is during the ionization process. The ionization and recombination process of electron due to solar radiation influence the occurrence of maxima and minima TEC. Bagiya et al. [13] have stated that the variation of vTEC is directly proportional to solar activity level where the vTEC values significantly higher with the increase of solar activity.

On the other hand, there are some other factors that contribute to rapid fluctuations in the ionospheric TEC values such as space weather disturbances. The occurrence of geomagnetic storms and associated ionospheric storms are mainly due to the interference between the high energy charged particles and the earth's magnetosphere. The interference causes large perturbations in the earth's magnetic field which excite the ions and increase the electron densities in the ionosphere. Thus, the daily planetary magnetic index (Dst) was designed to describe variation in the geomagnetic field caused by these irregular current systems. It provides a daily average level for geomagnetic activity and the index is average of the eight ap values [14]. This index was obtained from the World Data Center for Geomagnetism, Kyoto (wdc.kugi.kyoto-u.ac.jp/kp/index.html)

2.2 Training Algorithm

Even though there are various training algorithms in FFD NNs which are applicable in many applications, generally it is difficult to determine which of these training algorithms will be most appropriate for different technical areas [7, 15-16]. Commonly, it depends on the architecture of the networks and the application areas.

In this study, the prediction of TEC is assessed among different training algorithms using Neural Network toolbox of MATLAB version 7.11.0.584. The training was done on a Intel Pentium 987 processor PC with the speed of 1.5 GHz and 4 GB memory under Windows7 operating system. The algorithms belong to four major methods: gradient descent (GDA: Batch gradient descent with variable learning rate; RP: Resilient back propagation), conjugate gradients (CGF: Fletcher - Reeves Update; CGB: Powell- Beale restarts; CGP: Polak-Ribiere Update; SCG: Scaled conjugate gradient), quasi-Newton (BFG: Boyden, Fletcher, Goldfarb and Shanno update quasi-Newton algorithm), and Levenberg-Marquardt (LM). A comprehensive discussion about the techniques and training algorithms is not included in this work.

The scope of this work will only include the performance of the training algorithms to develop an optimized TEC model and the desirable results. A common network structure is used, comprising an input layer with 6 input nodes (SSN, Ap, HRs, HRc, DNs, DNc), a single hidden layer and an output layer with 1 node to represent the TEC for all the training algorithms. Other than the precise inputs, the initial weights and biases in the network do contribute in the successive of the designed NN models [17]. Thus, each of the network training algorithms was run ten trials individually with random initialization of weights and biases to attain the best performance criteria and the results were used for comparison.

a. Performance criteria

The predictive ability assessment requires evaluation of data excluded from the training set. Hourly TEC data for month of July 2005 considered a truly independent dataset from the training which was used to verify the performance of the model.

In order to determine the best training algorithm in estimation of TEC, the correlation coefficient (R^2) of the linear regression line between the predicted value (NN TEC) and the observed value (GPS TEC) is taken into consideration and compared in terms of capability. The R^2 measures the explanatory or predictive power of a regression model. It is a goodness-of-fit measure, indicating how well the linear regression equation fits the data. It takes on value between the interval [0, 1] and the greater the value is; the better the designed model fits the observational data well.

Secondly the training period in the NN is assessed to study the training of the networks. The comparisons between the computational times required by the eight algorithms to train the networks are also used as a performance criterion to evaluate the networks.

3. Results and Discussions

Fig. 1 shows the training period and the R^2 results of the TEC prediction for each of the training algorithms. The computational time is very important in NN applications. The training period is measured for each of the training algorithm and their computational periods were in the order of: LM > RP > GDA > SCG > CGB > CGP > CGF > BFG. It is clearly seen that the LM training algorithm has the fastest convergence (~ 13.050s) with less number of iterations. Despite the fact LM training algorithm requires more memory and high computational complexity yet the algorithm yielded the least computation time [18]. The computational time of conjugate gradient algorithms are generally higher than other training algorithms. Mostly conjugate gradient requires slightly higher storage compared to other methods, thus it takes a longer time to converge.

From the correlation coefficient results, it is found that the R^2 value was above 0.9 for all the eight training algorithms. Among the algorithms, the conjugate gradient especially the model which was trained by CGB training algorithm (R^2 = 0.9568) method shows the highest predictive performance. The lowest R^2 value is obtained for SCG training algorithm (R^2 = 0.9458) indicating that the predictability of this training function was not as good as the CGB. The deviation of the R^2 value among the eight training algorithms was small and the measurement only differs in the range of 0.004 to 0.0044 from the highest correlation coefficient.

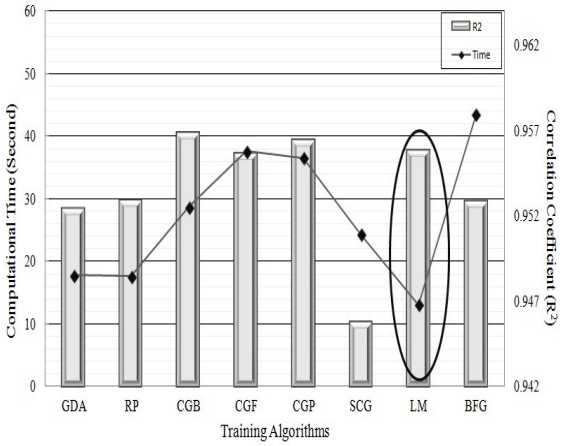

Figure 1: Comparison of training time and correlation coefficient for each training algorithm

The evaluation of the training performance of the networks for different training algorithm according to computational time and R^2 is shown in the figure above. In this study, the best prediction result is obtained with the LM training algorithm. LM was selected even though the training algorithm has smaller R^2 (R^2 = 0.9558) value than the gradient descent method. This is mainly because according to Ghaffari et al. [16], the R^2 evaluate the robustness of a model, where a model that provides correlation coefficient more than 0.9 is considered a well trained model and has high goodness of fit.

Furthermore, in real application, a high performance algorithm with the fastest convergence is needed. A longer computational time on the training data is likely to lead to over fit or over generalization, where the model could adopted random noises and deteriorate the performance of the designed model. In this study it can be seen, amongst various training algorithm the LM training method is found to have the fastest convergence rate. The superiority of LM is proven in this study and makes it more preferable algorithm in ionospheric TEC prediction application.

4. Summary

Training performance of eight training algorithms in neural network in predicting the ionospheric TEC was investigated and compared. Computational time and R^2 were used as the performance criteria to evaluate the training performance. The LM algorithm has shown the fastest convergence rate and high correlation coefficient. The speed and goodness-of-fit in TEC prediction advantages make the LM more preferable algorithm in ionospheric prediction application

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Speed Performance Improvement of BLDC Motor for Electric Vehicle Using PID Control

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Abstract - Brushless Direct Current (BLDC) motor drives is continually gaining popularity in industrial and home appliance applications. Therefore, it is necessary to have a low cost, but effective BLDC motor speed/torque regulator. This paper presents a PID controller to get higher speed performance of BLDC for electric vehicle application. The PID constants determined using Ziegler-Nichols method. By this PID constants, PID controller results the voltages that entrance in the Pulse Width Modulation (PWM) circuit, and then produce the pulse triggers of the power inverter in certain duty cycle. Simulation results using Matlab/Simulink show that PID controller can improve the speed regulation and acceleration of motor speed.

1. Introduction

Brushless Direct Current (BLDC) motor is one of the new design concepts of permanent magnet motor. This motor has been developed since late of 1980's. BLDC motors have a trapezoidal back-EMF. Another permanent magnet brushless motor is a sinusoidal back-EMF waveform and usually called Brushless Alternating Current (BLAC). In modern electrical machines industry productions, the BLDC motors are rapidly gaining popularity. BLDC motors are used in industries such as Appliances, electric vehicles, HVAC industry, medical, aircrafts, military equipment, hard disk drive, etc.

BLDC motors have high starting torque capability and smooth speed control like DC motor, but BLDC motors more efficient than DC motors and AC motors. The other advantages of BLDC motors are low maintenance, high power density, no brush sparking and high operating speeds. BLDC motors are more expensive due to their complex control system, the use of rare earth magnets and the need for rotor position sensors. But this price is overcome by a longer life and lower maintenance costs.

BLDC controls convert Hall signals or back-EMF signal to six trigger signals. Frequently, this control built using microcontroller. In the electric vehicle applications, especially for race electric vehicle, the motor control need to improve acceleration, speed and deceleration. In this research, a simple BLDC control will be improve using PID controller. The PID constant determine using Ziegler-Nichols tuning. To verify the proposed control, a modeling of BLDC motor and control for electrical vehicle build on Matlab-Simulink circuit [8-10].

2. Material and Method

2.1 Motor Structure

BLDC motors that also referred to as an electronically commutated motor is not have brushes. The commutation is performed electronically at certain rotor positions. Most of BLDC motors are driven by rectangular or trapezoidal voltage coupled with the given rotor position. The voltage must be properly aligned between the phases, so that the angle between the stator flux and the rotor flux is kept close to 90 to get the maximum developed torque. BLDC motors often incorporate either internal or external Hall position sensors to sense the actual rotor position [1-5]. Fig. 1 shows general structure of BLDC.

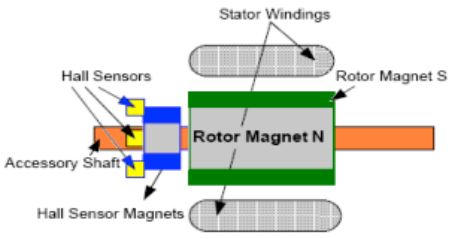


Fig.1 The Structure of BLDC Motor

2.2 Modelling

Based on BLDC modelling as shown in Fig.2 and if assume the BLDC motor produce trapezoidal back EMF e_a, e_b and e_c, the waveform of exited current i_a, i_b and i_c is rectangular. If all the series resistance winding assume is R_s and the self and mutual inductance L_s, the electric equation of BLDC motor is shown in equation (1) [1-7].

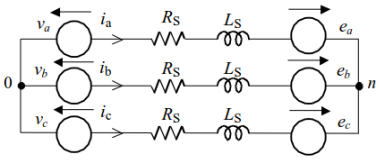


Fig. 2 Electrical model of BLDC motor [1-5]

$$\begin{bmatrix} v_{an} \\ v_{bn} \\ v_{cn} \end{bmatrix} = \begin{bmatrix} R_s & 0 & 0 \\ 0 & R_s & 0 \\ 0 & 0 & R_s \end{bmatrix} \begin{bmatrix} i_a \\ i_b \\ i_c \end{bmatrix} + \begin{bmatrix} L_s - M & 0 & 0 \\ 0 & L_s - M & 0 \\ 0 & 0 & L_s - M \end{bmatrix} \frac{\mathrm{d}}{\mathrm{d}t} \begin{bmatrix} i_a \\ i_b \\ i_c \end{bmatrix} + \begin{bmatrix} e_a \\ e_b \\ e_c \end{bmatrix} \quad (1)$$

The interaction magnetic field on the winding and permanent magnet, produce electromagnetic torque as shown in equation (2), where ω_n is the mechanical speed of rotor of BLDC. Therefore the equation motion of this motor is shown in equation (3), where T_L is load torque, B is damping constant and J is moment inertia of shat and load [1].

$$T_e = \frac{e_a i_a + e_b i_b + e_c i_c}{\omega_m} \quad (2)$$

$$\frac{\mathrm{d}\omega_m}{\mathrm{d}t} = \frac{T_e - T_L - B\omega_m}{J} \quad (3)$$

In the six step BLDC control, the power will be deliver from two phases as shown in equation (4), where I is the current amplitude and E is amplitude of back-EMF. Using equation (2) and (4), the electric torque can also express as equation (5), where k_t is motor torque constant [1,3,5].

$$P_o = \omega_m T_e = 2EI \quad (4)$$

$$T_e = 2k\phi I = 2k_t I \quad (5)$$

2.3 BLDC Control System

Complete control system of BLDC is shown in Fig. 3. This system consists of inverter (1), driver circuit (2), controller (3), Hall effect sensors (4), and BLDC motor (5), [2,4,6]. Hall sensors (4) give sign of position the permanent magnet in the windings. These positions generally indicate using three bits data. These data then entered in a control circuit (3) to convert in the 6 trigger signals for inverter (1). The inverter composed from power device like MOSFET or IGBT. In the control circuit, the trigger signals also regulate to desire direction and speed of rotation. Before enter to the inverter, the trigger signals entered to the driver circuit (2). In this circuit, the signals will be matching to the switch devices. Finally, by condition of switch of inverter, the power flow to windings. Hall sensor detects new position and the process will be return to control circuit [7].

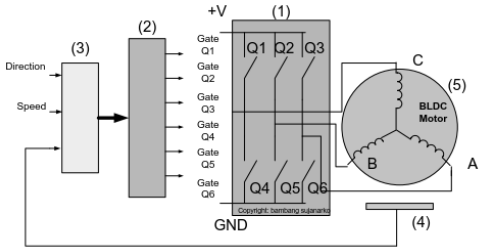


Fig.3 BLDC control system [6,7]

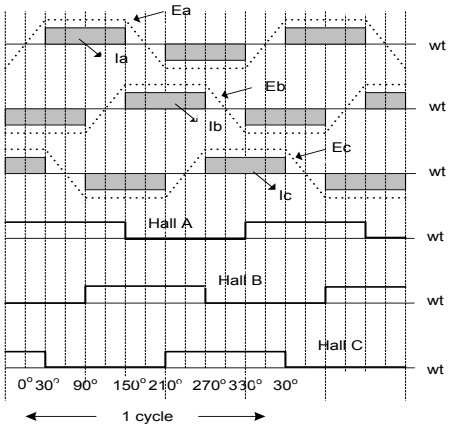


Fig.4 Back-EMFs, current and Hall position sensors waveform of BLDC motor [6,7]

Table 1. Relationship among direction, the position of Hall sensor and switching in the inverter [2,4,6.7]

	Dir.	H_C	H_B	H_A	Q_1	Q_2	Q_3	Q_4	Q_5	Q_6
CW	1	1	0	1	1	0	0	0	1	0
	1	1	0	0	0	0	1	0	1	0
	1	1	1	0	0	0	1	1	0	0
	1	0	1	0	0	1	0	1	0	0
	1	0	1	1	0	1	0	0	0	1
	1	0	0	1	1	0	0	0	0	1
CCW	0	0	0	1	0	0	1	1	0	0
	0	0	1	1	0	0	1	0	1	0
	0	0	1	0	1	0	0	0	1	0
	0	1	1	0	1	0	0	0	0	1
	0	1	0	0	0	1	0	0	0	1
	0	1	0	1	0	1	0	1	0	0

BLDC control produce six different pattern in one cycle. Because there are six pattern of commutation, so it called six step commutations. The signal pattern shown in Fig. 4. This figure show the waveforms of Back-EMFs (E_a, E_b, E_c), current (I_a, I_b, I_c) and Hall position sensors (Hall A, Hall B and Hall C). Back-EMFs in this figure is the trapezoidal type, other type of Back-EMFs is sinusoidal. In this figure, the first commutation occurs in 30° until 90°, the 2th in 90° until 150°, the 3th in 150° until 210°, the 4th in 210° until 270°, the 5th in 270° until 330° and the 6th

in 330° until 30° [7]. The relationship among waveform can be expressed using Table 1. Using Karnaugh-Map simplification, this table produces a simple logic function in equation (6)-(11) [7].

$$Q_1 = \mathrm{Dir}\overline{\mathrm{H_B}}\mathrm{H_A} + \overline{\mathrm{Dir}}\mathrm{H_B}\overline{\mathrm{H_A}} \quad (6)$$

$$Q_2 = \mathrm{Dir}\overline{\mathrm{H_C}}\mathrm{H_B} + \overline{\mathrm{Dir}}\mathrm{H_C}\overline{\mathrm{H_B}} \quad (7)$$

$$Q_3 = \mathrm{Dir}\mathrm{H_C}\overline{\mathrm{H_A}} + \overline{\mathrm{Dir}}\overline{\mathrm{H_C}}\mathrm{H_A} \quad (8)$$

$$Q_4 = \mathrm{Dir}\mathrm{H_B}\overline{\mathrm{H_A}} + \overline{\mathrm{Dir}}\overline{\mathrm{H_B}}\mathrm{H_A} \quad (9)$$

$$Q_5 = \mathrm{Dir}\mathrm{H_C}\overline{\mathrm{H_B}} + \overline{\mathrm{Dir}}\overline{\mathrm{H_C}}\mathrm{H_B} \quad (10)$$

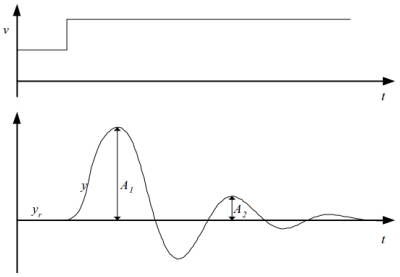
$$Q_6 = \mathrm{Dir}\overline{\mathrm{H_C}}\mathrm{H_A} + \overline{\mathrm{Dir}}\mathrm{H_C}\overline{\mathrm{H_A}} \quad (11)$$

In a BLDC, there are two phases simultaneously active with square wave excitation and in the magnet distance approximately equal to the pole arc, the electric torque (T_e) is given as equation (12) [6,7]. Substitute equation (12) to (2), the angular speed of BLDC motor can be arranged into equation (13). If V produce from voltage supply (V_s) using PWM method with D duty cycle, the voltage (V) can calculate using equation (14) [6,7] and finally become equation (14).

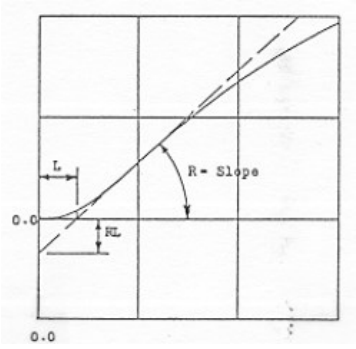
$$T_e = N_p\ N_t\ N_{spp}\ P\ I\ B_r\ L_x\ R_x \quad (12)$$

$$\omega \cong \mathrm{k}\frac{3EI}{N_p\ N_t\ N_{spp}\ P\ I\ B_r\ L\ R} \cong kE \cong kV \quad (13)$$

$$\omega \cong kDV_s \quad (14)$$



(a) Open loop respond



(b) Parameter

Fig.5 Open loop responds of system that accepted using Zieger Nichols tuning.

2.4 PID Controller Ziegler-Nichols Tuning Method

To improve BLDC speed in the better respond and stability, BLDC controller equipped with Proportional Integral Derivative (PID) controller. PID Constanta can determine using various methods. Among these methods, Zieger-Nichols method is the simplest. Ziegler-Nichols have two types' method, i.e. the closed loop method and open loop method. Tuning of Zieger Nichols accepted in stability region, if the ratio of the amplitudes of subsequent peaks in the same direction (A_2/A_1) is approximately 1/4. Fig.5 show open loop respond of system that accepted using Zieger Nichols tuning and Table 2 show Formula parameter of PID controller using Zieger Nichols [7].

Table 2. Formula parameter of PID controller using Zieger Nichols.

	K_p	T_i	T_d
P controller	$\frac{1}{LR/U}$	∞	0
PI controller	$\frac{0.9}{LR/U}$	$3.3L$	0
PID controller	$\frac{1.2}{LR/U}$	$2L$	$0.5L = \frac{T_i}{4}$

2.5 BLDC Modellingand Simulation Circuit

If back-EMF of BLDC assume have 120 degree phase shift with respect to each other and all phase current is equal, so based on equation (1) until (3), the state space BLDC is shown in equation (15) and (16) [7]. Implementing these state space equations will make model more complex, therefore these equation is divided to two simple models, that is electrical and mechanical model. Using Simulink Matlab these model shown in Fig.6 and Fig.7.

$$\begin{bmatrix} i_a' \\ i_b' \\ \omega_m' \end{bmatrix} = \begin{bmatrix} -\frac{R}{L} & 0 & 0 \\ 0 & -\frac{R}{L} & 0 \\ 0 & 0 & -\frac{\beta}{J} \end{bmatrix} \begin{bmatrix} i_a \\ i_b \\ \omega_m \end{bmatrix} + \begin{bmatrix} \frac{2}{3L} & \frac{1}{3L} & 0 \\ -\frac{1}{3L} & \frac{1}{3L} & 0 \\ 0 & 0 & \frac{1}{J} \end{bmatrix} \begin{bmatrix} V_{ab} - E_{ab} \\ V_{bc} - E_{bc} \\ T_e - T_l \end{bmatrix} \quad (15)$$

$$\begin{bmatrix} i_a \\ i_b \\ i_c \\ \omega_m \end{bmatrix} = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ -1 & -1 & 0 \\ 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} i_a \\ i_b \\ \omega_m \end{bmatrix} \quad (16)$$

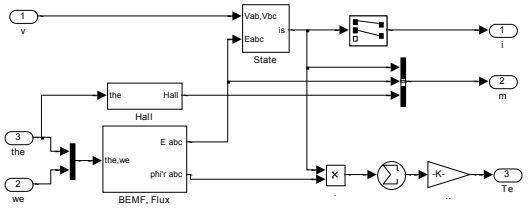


Fig.6 Electrical Simulink Model of BLDC [7]

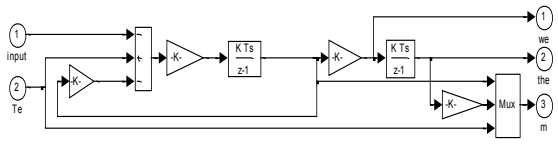


Fig.7 Mechanical Simulink model of BLDC [7]

Using load of electric vehicle modeled in the step simulink block, inverter on Universal Bridge and PID controller to improve performances, the simulation circuit of BLDC motor for electric vehicle is shown in Fig.8.

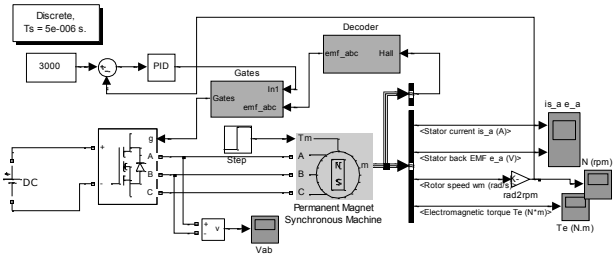


Fig. 8 Simulation circuit of BLDC motor for electric vehicle using PID controller

3. Result and Discussion

Performance improvement verified in the speed acceleration, speed in the increase load, decrease load and in the speed deceleration.

3.1 Speed Accelaration

To verify speed acceleration the system tested using constan load 1 Nm. The motor accelerated from 0 to 3000 rpm. Result simulations show that in the system without PID controller the rotary speed can not equal to setting speed, the actual speed above of setting speed. But if the system using PID controller, the rotary speed is stable in the set point. The result of this test ilustrated in the Fig. 9. Only a few different between with and without PID controller in the speed acceleration. PID controller can increase acceleration/

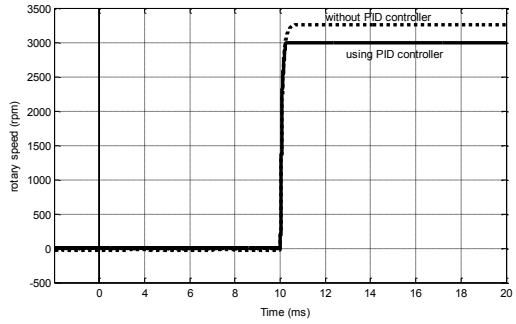


Fig. 9 Simulation result of speed acceleration

3.2 Speed on Increase Load

Rotary speed of motor in the without PID controller system will be decrease if the load of the BLDC system is increase. But if the system used PID controller, the rotary speed is constant and equal to setting point. Fig. 10 shows this phenomenon. The PID controller can result speed stabilization of BLDC motor for electric vehicle.

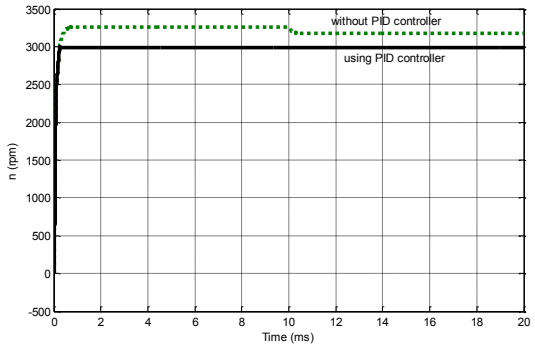


Fig. 10 Simulation result rotary speed of increase load

3.3 Speed in The Decrease Load

In the decrease load and the system without PID controller, BLDC motor speed will be increase. But if the system used the PID controller, the speed motor is constan as setting poind. Fig 11 shows different result of the system in the 2000 rpm, and the load cecrease from 2Nm to 1 Nm.

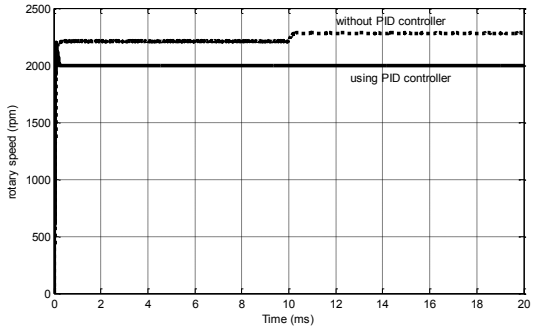


Fig. 11 Simulation result rotary speed of decrease load

3.4 Speed Deceleration

Deceleration performance of BLDC motor whitout the manual braking only depend on the load. PID controller used not influence the decelaration performance. If the system use high load, the decelaration is stop rapidly, otherwise if the system used light load the system stop in the longer time.

Fig. 12 show the result rotary speed in the system on the differend load and without or using PID controller. So deceleration of electric vehicle only depend of the breaking system.

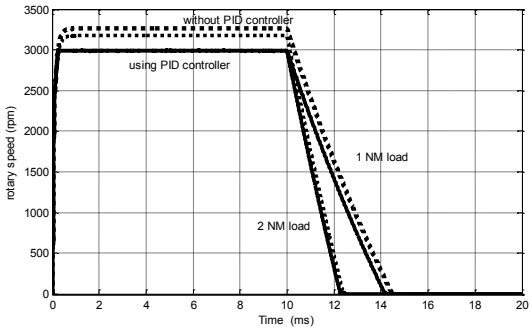


Fig. 12 Simulation result of speed deceleration

4. Summary

This paper presented simulation result of PID used in the BLDC motor for electric vehicle. PID controller used can improve acceleration and speed regulation in the variable load, but not in the deceleration. The deceleration more influenced by load and breaking system. This result can used to design electric vehicle control for race, where the acceleration, speed regulation in the various load and race tracks is crucial.

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In Vitro Inhibitory Activity of *Atuna racemosa, Euphorbia hirta and Diplazium* esculentum Juices Against α-Amylase and α-Glucosidase

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Abstract **-** Diabetes mellitus is one of the chronic diseases affecting millions of people worldwide. One way to manage it is to delay the breakdown of carbohydrates by inhibiting the action of enzymes. The α-amylase and α-glucosidase inhibition of these selected terrestrial flora are tested because they showed potential hypoglycemic activity in unpublished studies. *Diplazium esculentum* (92.090% ± 0.192) exhibited the highest α-amylase enzyme inhibition activity followed by *Euphorbia hirta* (91.711% ± 0.234), and *Atuna racemosa* (33.503% ± 6.238). *D. esculentum* (70.016% ± 2.359) exhibited the highest α-glucosidase enzyme inhibition activity followed by E. hirta (69.588% ± 2.269) and *A. racemosa* (11.815% ± 6.908). The activities of the groups against the two enzymes are significantly different ($p < 0.01$) from that of Acarbose (standard drug). Flavonoids, phenol, saponins, steroids and tannins are found in all samples while alkaloids are present only in *D. esculentum* and *E. hirta*.

1. Introduction

According to the World Health Organization (2011) chronic diseases, such as heart disease, stroke, cancer, obstructive pulmonary diseases and diabetes, are by far the leading causes of mortality in the world, representing 63% of all deaths. A total of 25.8 million children and adults in the United States (8.3% of the population) have diabetes (American Diabetes Association, 2013).

One out of every five adult Filipinos are diabetic, according to the latest national survey conducted on the prevalence of diabetes in the country. The survey, conducted in 2007 by the Philippine Cardiovascular Outcome Study on Diabetes Mellitus further shows that as many as three out of five adults are already diabetic or on the verge of developing diabetes unless they change their lifestyle. The latest survey shows that 20.6% of the adult population (ages $\geq$ 30) suffer from diabetes.

Diabetes mellitus is a serious metabolic disorder characterized by defects in the body's use of carbohydrates. It is characterized by an increase in blood sugar levels associated with long term damage and failure of organ functions, especially the eyes, kidneys, nerves, heart and blood vessels. Type 1 diabetes results from inadequate synthesis of insulin by β-cells of the pancreas, while type II diabetes is characterized primarily by insulin resistance (a condition in which peripheral cells do not respond normally to insulin) or β-cell dysfunction [1]. It occurs when the pancreas does not adequately produce insulin or the body cannot properly utilize insulin. Inhibitors of α-amylase and α-glucosidase delay the breaking down of carbohydrates in the small intestine and diminish rapid increase of postprandial blood glucose.

In the days when drugs from the West just started to be used in the Philippines, they were not only too expensive but also too scarce. The rural folks and the poor continued to rely on herbal medicines available in abundance, locally. Because of this situation, "many researchers in the Philippines are testing edible plants for their anti-diabetic activity and other therapeutic activities related to diabetes. A popular vegetable in the Philippines, *Amplaya,* has the most documented scientific reports since 1960's citing its blood sugar-lowering benefit.

The possibility of such activity may also exist in other terrestrial plants in the Philippines hence the anti-diabetic activity of these three terrestrial floras: *Atuna racemosa, Euphorbia hirta* and *Diplazium esculentum* are studied. These plants are chosen for the following reasons: (1) popularity : *Atuna racemosa* fruit is used as a condiment in a Filipino dish, *Euphorbia hirta* leaves are used as tea infusion to treat dengue and *Diplazium esculentum* fronds are eaten as vegetable salad; (2) the selected plants are abundant in the Philippines (native plants); (3) unpublished studies of these plants showed potential hypoglycemic activity: Castillones and Fernandez (2012), Jayme and Vidal (2012), Rone and Tan (2010), all conducted at the Department of Pharmacy, University of San Carlos, Cebu City.

However, none of those studies could explain the possible mechanism of action of the anti-diabetic activity of the selected plants. Thus this study

methodology tested the inhibition of α-amylase and α-glucosidase enzymes which are responsible for the breakdown of carbohydrates to glucose. Inhibiting these enzymes causes slowing of breakdown of carbohydrate to glucose that helps keep blood glucose from rising very high after meals. Lastly, the methodology does not involve animal sacrifices as a preliminary screening of anti-diabetic activity of the selected plants.

2. Materials and Methods

2.1 Collection and Preparation of Samples

Fresh whole fruits *Atuna racemosa* were obtained from Ozamiz City. The fruit were washed thoroughly and were cut into half using aknife. The seed were separated from the peel using a spoon. Fresh leaves of *Euphorbia hirta* were collected from Cebu city. The leaves were washed thoroughly and were cut into fine pieces. Fresh fronds of *Diplazium esculentum* were procured from Dumaguete city. The fronds were washed thoroughly and were cut into fine pieces.

2.2 Preparation of Pure Juice

Five hundred gram-portions of each fresh plant sample was used in the extraction of the juice with the aid of a juicer.

2.3 Secondary Metabolite Screening

A portion of each juice was subjected to thin layer chromatography to determine the constituents present in each plant sample.

2.4 Chemicals

All chemicals and reagents used were of the highest commercially available purity. They were purchased from Sigma-Aldrich through the Department of Pharmacy, USC.

2.5 Inhibition of α-amylase Enzyme Assay

A total of 500 µl of the pure juices or standard drug (Acarbose) was added to 500 µl of 0.2 M phosphate buffer (pH 6.9) containing α-amylase (0.5mg/ml) solution and was incubated at 25°C for 10 min. After these, 500 µl of a 1% starch solution in 0.2 M phosphate buffer (pH 6.9) was added to each tube. The reaction mixture was incubated at 25°C for 10 min. The reaction was stopped with 1.0 ml of 3, 5 dinitrosalicylic acid color reagent. The test tubes were incubated in boiling water bath for 5 min then cooled to room temperature. The reaction mixture was diluted with 10 ml distilled water and absorbance was measured at 540 nm. The control represents 100% enzyme activity and was conducted in similar way but without the pure juices or standard drug [2-3].

2.6 Inhibition of α-glucosidase Enzyme Assay

The inhibitory activity was determined by incubating a solution of 333 µl 2% maltose with 333 µl 0.2 M Tris buffer (pH 8.0) and 333 µl of pure juices or standard drug (Acarbose) for 5 min at 37°C. The reaction was initiated by adding 1ml of α-glucosidase enzyme (1 mg/ml) to it followed by incubation for 10 min at 37°C. Then the reaction mixture was heated for 2 min in boiling water bath to stop the reaction. One ml of each resulting test solution was added 2 ml glucose oxidase/peroxidase reagent. The solution was incubated for 30 minutes at 37°C. The reaction was stopped by adding 2 ml of 12 N H2SO4. The absorbance was measured at 540 nm. Control represent 100% enzyme activity and was conducted in similar way but without the pure juices or the standard drug [2-3].

2.7 Computation of Percent Inhibition

Percentage α-amylase and α-glucosidase enzyme inhibition activity was calculated by:

$$I\ \% = [(Ac-As)/Ac]*100 \quad (1)$$

where I % is the percent enzyme inhibition, Ac is the absorbance of the control and As is the absorbance of the sample (juice/standard drug) [2-3].

2.8 Statistical Analysis

All samples were tested in triplicates. The means ± SD (standard deviation) of all results were calculated. Statistical analysis was done using one way analysis of variance using SPSS, Version 20. A significant difference was judged to exist at a level of $P < 0.01$.

3. Results

3.1 Secondary Metabolite Screening

The secondary metabolites that tested positive in the different samples were flavonoids, phenol, saponins, steroids and tannins (Table 1).

Table 1: Secondary Metabolites Screening

Secondary Metabolites	Pure Juice		
	Atuna racemosa	*Euphorbia hirta*	*Diplazium esculentum*
Alkaloids	-	+	+
Anthraquinones	-	+	-
Flavonoids	+	+	+
Phenol	+	+	+
Saponins	+	+	+
Steroids	+	+	+
Tannins	+	+	+

3.2 Inhibition of α-amylase Enzyme

Figure 1 shows the absorbance (nm) of each test groups containing different pure juices after the α-amylase enzyme inhibition assay. The control group showed the highest absorbance (2.633 ± 0.053) followed by *Atuna r*acemosa (1.752 ± 0.179) while

Acarbose (0.069 ± 0.004) showed the lowest absorbance.

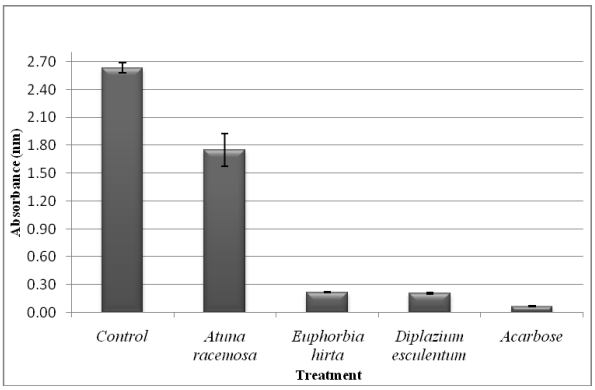

Figure 1: α-amylase Enzyme Inhibition Activity

Figure 2 shows the percent α-amylase enzyme inhibition activity of each test groups containing different pure juices. Acarbose (97.384% ± 0.134) showed the highest activity followed by *Diplazium esculentum* (92.090% ± 0.192) while *Atuna racemosa* (33.503% ± 6.238) showed the lowest activity.

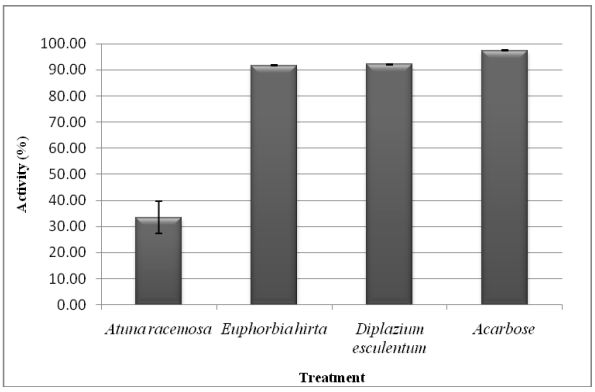

Figure 2: α-amylase Enzyme Inhibition Activity

3.3 Inhibition of α-glucosidase Enzyme

Figure 3 shows the absorbance (nm) of each test groups containing different pure juices after the α-glucosidase enzyme inhibition assay. The control group (1.514 ± 0.114) showed the highest absorbance followed by *Atuna racemosa* (1.330 ± 0.090) while Acarbose (0.202 ± 0.006) showed the lowest absorbance.

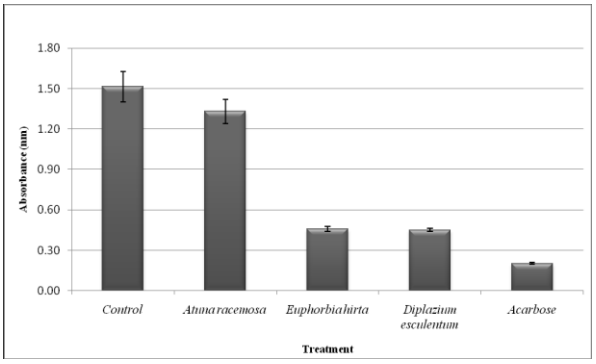

Figure 3: α-glucosidase Enzyme Inhibition Activity

Figure 4 shows the percent α-glucosidase enzyme inhibition activity of each test groups containing different pure juices. Acarbose (86.576% ± 0.793) showed the highest activity followed by *Diplazium esculentum* (70.016% ± 2.359) while *Atuna racemosa* (11.815% ± 6.908) showed the lowest activity.

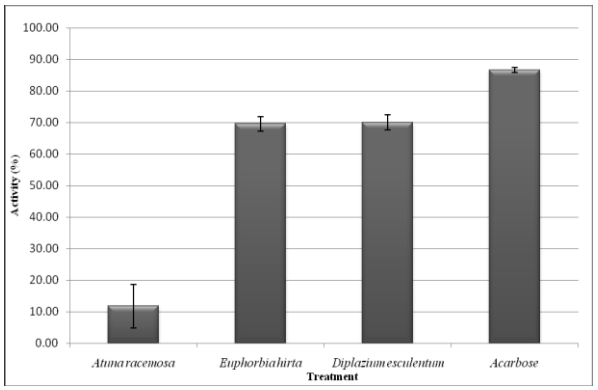

Figure 4: α-glucosidase Enzyme Inhibition Activity

4. Discussion

The samples showed anti-diabetic activity by inhibiting the action of both α-amylase and α-glucosidase enzymes. Among the three, however, *Atuna racemosa* exhibited the lowest α-amylase (33.503% ± 6.238) and α-glucosidase (11.815% ± 6.908) enzyme inhibition activity. *Euphorbia hirta* exhibited α-amylase (91.711% ± 0.234) and α-glucosidase (69.588% ± 2.269) enzyme inhibition activities while *Diplazium esculentum* exhibited the highest α-amylase (92.090% ± 0.192) and α-glucosidase (70.016% ± 2.359) enzyme inhibition activities. The enzyme inhibition activites of *E. hirta* and *D. esculentum* are, however, significantly different ($p < 0.01$) from the enzyme inhibition activity of Acarbose (standard drug).

Many plants have been studied for their hypoglycemic potential [4-5]. The secondary metabolites from these plants could have played a significant role in such activity either acting alone or in synergy with the others. This includes alkaloids [6-8],

flavonoids [9-10], phenolics [11-12] and triterpenoids [13].

Based on the phytochemical screening test, only *A. racemosa* did not give positive result to alkaloid test (all exhibited positive result to flavonoids, phenol, saponis, steroids and tannins). This result could have a bearing on its low enzyme inhibition compared to the other two samples [14]. Alkaloids together with anthraquinones, flavonoids, steroids, terpenoids and cardiac glycosides showed some restoration action on the β-cell which results into inhibition of aldose reductase activity, which is beneficial in mitigating the glucose autoxidation, glycation and acts against the major contributor reactive oxygen species (ROS) and other free radical. This would lead to regeneration of β-cells and led to the normal glucose level [15].

The result shown by the three samples is also validated by the results of previous studies which mentioned flavonoids, phenols, saponins, steroids, alkaloids and terpenoids as the possible secondary metabolites which could be responsible for the action of the test extracts to effectively inhibit α-amylase [16]. The positive relationship between the phenols and flavonoid content and the ability to inhibit intestinal α-glucosidase and pancreatic α-amylase of the samples is also documented in previous studies [17-18].

The findings in this study could pave the way for further studies such as isolating the metabolites of the three samples and testing each for their inhibitory effect on these enzymes.

5. Conclusion

The juices from the three plant samples showed both α-amylase and α-glucosidase enzyme inhibition activities. *Diplazium esculentum* (92.090% ± 0.192) exhibited the highest α-amylase enzyme inhibition activity followed by *Euphorbia hirta* (91.711% ± 0.234), and *Atuna racemosa* (33.503% ± 6.238). The activities of the groups are significantly different ($p < 0.01$) from Acarbose (standard drug).

Diplazium esculentum (70.016% ± 2.359) exhibited the highest α-glucosidase enzyme inhibition activity followed by *Euphorbia hirta* (69.588% ± 2.269) and *Atuna racemosa* (11.815% ± 6.908). The activities of the groups are significantly different ($p < 0.01$) from Acarbose.

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Inhibitory Effects of Theobromine, a Cocoa Methylxanthine, of Platelet Aggregation and Adhesion Stimulated by Various Agonists using Modified 96-well Platelet Aggregometry

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Abstract - Previous studies have suggested cardioprotective effects of cocoa. We have evaluated the effects of theobromine, a cocoa methylxanthine on platelet aggregation and adhesion. Human platelet-rich plasma was incubated with theobromine (2-50µg/ml). Changes in absorbance in the presence or absence of various agonists, as a measure of platelet aggregation, were then determined at 595nm every 15s for 16min followed by evaluation of platelet adhesion. In this study, theobromine displayed concentration dependent inhibitory effects upon platelet activation in response to ADP, arachidonic acid, adrenaline, collagen and TRAP-6 but not to thromboxane-mimetic agent, U46619 or ristocetin stimulation. Our data demonstrate that theobromine inhibits platelet activation in response to some but not all agonists. These effects may due to the inhibition of platelet phosphodiesterase, increasing platelet levels of cyclic AMP and cyclic GMP and so reducing platelet reactivity.

1. Introduction

High antioxidant diet has been shown to have influence on cardiovascular disease. *Theobroma cocoa* beans, is constitute with estimated 380 identified chemicals and about 10 psychoactive compounds [1]. Consumption of dark chocolate may have an impact on cardiovascular disease via a number of mechanisms. The mechanism is not limited to certain target molecules such as lipid from oxidative damage, but also suppression of inflammation and modulation of vascular homeostasis [2]. The benefits of cocoa are suggested according to the low prevalence of atherosclerotic disease, hypertension, diabetes and dyslipidemia in Kuna Indians of an island in the Coast of Panama. Previous study showed that there is decrease of blood pressure with significant improve of insulin response in healthy volunteers suggesting protective effects on the vascular endothelium [3].

Methylxanthines can be found in coffee, cocoa products and cola soft drinks. Theobromine (3,7-dimethylxanthine) is naturally occurring alkaloids that can be found in chocolate, tea and cocoa products [4]. Dark chocolate contains higher theobromine, 240-520 mg, as compared to 65-160 mg in milk chocolate per 50-g portion [5]. It is a metabolite of caffeine with dark chocolate contains the highest theobromine concentration in relative to caffeine that present in small amounts in dark chocolate [6].

Platelet plays a major role in the pathophysiology of atherosclerosis through their effects on inflammation. Despite their roles in modulating inflammation, platelets are directly involved in thrombosis and subsequent acute vascular events including acute coronary syndromes, ischaemic strokes and symptomatic peripheral arterial disease. Under pathological conditions, platelet aggregate formation, i.e. a thrombus, rapidly occurs within vasculature as a response to events such as plaque rupture [7]. This study evaluated the *in vitro* effects of theobromine on platelet aggregation and adhesion stimulated by various agonists using modified 96-well plate aggregometry.

2. Materials and Methods

2.1 Preparation of platelet-rich plasma

Human platelets were obtained from whole fresh blood drawn from healthy volunteers who had not consumed any medication for 10 days prior to the test. Human blood was collected by venepuncture into tri-sodium citrate (3.2% w/v) final. Human platelet-rich plasma (PRP) was obtained from blood by centrifugation at 175 x g for 15 minutes at room temperature. Platelet counts were checked to ensure they were within the normal range of 150-400 x 10^{-3}/L. PRP is further centrifuged at 15 000 x g for 5 min to obtain platelet-poor plasma (PPP). PRP was incubated for 30 minutes, at 37^0C with theobromine treatment (2, 5 and 10 µg/ml; Sigma, UK).

2.2 Measurement of platelet aggregation

Platelet aggregation and adhesion were performed as described previously [8]. Theobromine

treated PRP (100µL) was then added to the wells of 96-well plates (Nunc; VWR, Lutterworth, Leicestershire, UK) in the presence or absence of platelet agonists. The platelet agonists used in this study were arachidonic acid (AA,0.03-1 mmol L^{-1}; Sigma),adenosine diphosphate (ADP, 0.1_30 µmol L^{-1}; LabMedics, Salford, Manchester, UK), collagen (0.1_30 µg L^{-1}; Lab-Medics), epinephrine (0.001_100 µmol L^{-1}; LabMedics), TRAP-6 amide (0.1_30 µmol L^{-1}; Bachem, St Helens, Merseyside, UK), U46619 (0.1_30 µmol L^{-1}; Cayman Chemical Company, Ann Arbor, MI, USA) or vehicle. Absorbance was determined at 595nm every 15s for 16min at 37^0C. Changes in absorbance were converted to percent aggregation by reference to the absorbances of PRP and PPP.

2.3 Measurement of platelet adhesion

Platelet adhesion was determined at the end of the platelet aggregation [9]. Plates were washed twice with 100µl of 0.9% saline. 100% adhesion controls were prepared by centrifugation of 500µl of PRP at 15 000 x g for 2 minutes. The plasma was discarded prior to resuspension of the pellet in Assay Buffer containing citric acid, 13.7 mmol L^{-1}; sodium citrate, 43.6 mmol $^{L-1}$; Triton X-100, 0.1% v/v; p-nitrophenol phosphate, 0.2 mg mL^{-1}; Sigma. 100µl of Assay Buffer was pipetted into each well before 15 minutes incubation. To stop the reaction, 100µl of 2M NaOH (Sigma, UK) was added into each well. Then, the plate was read at 405nm using a microplate reader. Background absorbance was substracted from these values. The percentage of adhesion was calculated using formula; (Absorbance of sample-Blank) / (Absorbance of control-Blank) x 100.

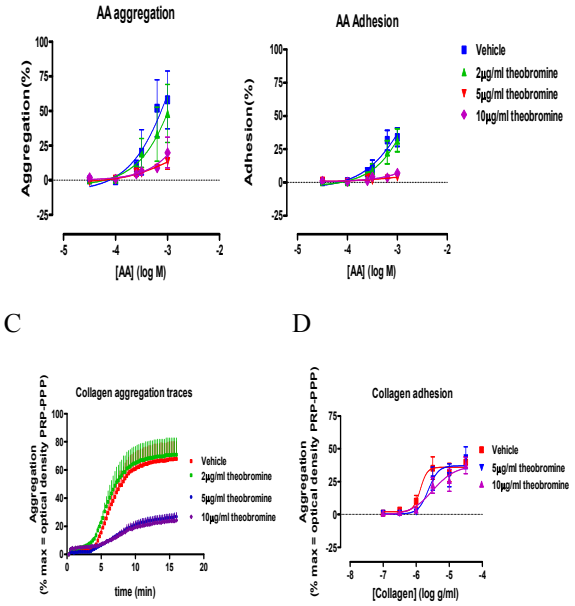
2.4 Statistical Analysis

All data were analysed using GraphPad Prism 4.0 and expressed as mean and standard error of mean (S.E.M.). Statistical analyses were performed when appropriate.

3. Results

3.1 Effects of theobromine on platelet aggregation

Our data shows that theobromine demonstrate various inhibition effects in concentration dependent of platelet function. For instance, platelet aggregation stimulated by 3µM ADP was decreased to 35±9% and 32±7% when incubated with 5 and 10µg/ml theobromine respectively as compared to control vehicle, 45±9% (Table 1). Platelet aggregation by 3µg/ml collagen, 69±13% was also reduced to 33±14% by 5µg/ml theobromine (Figure 1). Incubation with theobromine also inhibited platelet aggregation and adhesion by arachidonic acid, adrenaline, and TRAP-6 but not to U46619 and ristocetin (data not shown).



A B C D

Figure 1: Theobromine inhibited platelet induced by arachidonic acid (AA); A) platelet aggregation; B) platelet adhesion; and collagen, C) platelet aggregation; D) platelet adhesion. Each value represents means ± S.E.M. (n=4).

3.2 Theobromine concentration response curve on ADP and TRAP-6 stimulated platelets

To determine if the effects of theobromine is concentration-dependent, treatment of theobromine ranging from 2-50µg/ml is performed in platelet induced by ADP and TRAP-6. Theobromine reduces platelet aggregation dependently on treatment concentration as compared to control. For an example, in 3µM ADP induces platelet, percentage of aggregation decreases from 45.8±10% in control to 32.6±7%, 33.8±9% and 26.4±7% following incubation with 10, 20 and 50µg/ml theobromine respectively (Figure 2). Platelet adhesion was also inhibited in a concentration-dependent manner, which shows the decrease as compared to control, 9.4±3%, to 7.6±2% and 5.0±2% with 20 and 30µg/ml theobromine, respectively. Similar observation was also found with TRAP-6-induced platelet (data not shown).

Aggregation (%)				
Agonist (log M)	Theobromine (µg/ml)			
	0	2	5	10
ADP				
-5.5	29.8±4	34.3±6	25.5±5	26.0±3
-5.0	59.3±6	60.8±7	53.5±3	52.3±3
-4.5	70.5±8	69.8±6	67.5±1	69.5±3
Adrenaline				
-6.0	23.5±14	20.3±10	4.0±2	9.8±3
-5.0	29.0±5	56.3±10	12.8±5	25.8±9
-4.0	41.8±12	62.0±10	27.3±10	40.3±13
TRAP-6				
-5.5	38.8±11	45.3±13	19.8±6	25.3±6
-5.0	79.8±10	83.3±7	81.3±5	84.0±4
-4.5	89.8±4	90.5±4	92.3±1	92.5±2
Adhesion (%)				
Agonist (logM)	**Theobromine (µg/ml)**			
	0	**2**	**5**	**10**
ADP				
-5.5	11.5±2	15.8±5	8.0±3	8.5±3
-5.0	22.5±5	22.3±7	18.3±5	17.5±5
-4.5	33.8±6	30.3±7	22.8±5	24.5±7
Adrenaline				
-6.0	11.5±8	13.0±8	0.8±0	7.0±4
-5.0	16.5±6	30.8±10	10.0±7	12.0±6
-4.0	24.0±7	31.5±8	9.5±5	18.8±9
TRAP-6				
-5.5	19.5±7	21.5±7	5.5±4	3.8±2
-5.0	36.8±7	39.3±8	37.8±9	36.8±10
-4.5	46.5±8	42.8±7	42.8±8	42.0±6

Table 1: Inhibitory effects of theobromine at 2,5 and 10µg/ml on platelet aggregation and adhesion stimulated by ADP, adrenaline and TRAP-6 (n=4).

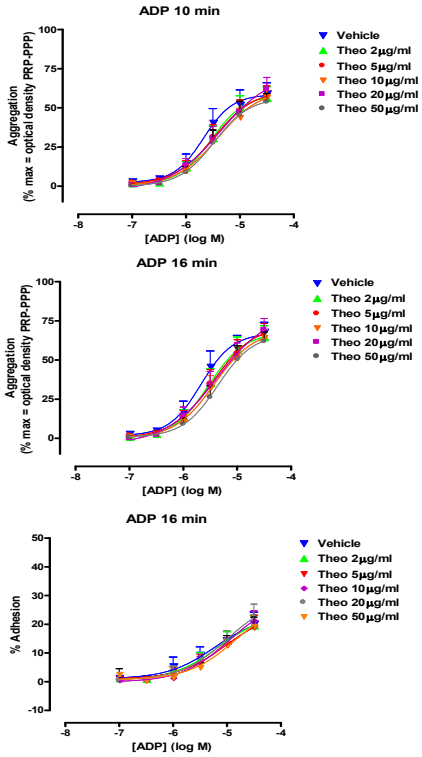


Figure 2: *Concentration response curve for platelet aggregation and adhesion of theobromine on ADP-stimulated platelet agonists.* PRP was incubated with theobromine at 2, 5, 10, 20 and 50µg/ml for 30 mins before platelet aggregation was measured in 96-well plate. Each value represent means ± S.E.M (n=5).

4. Discussion

Although limited, there are few studies of other methylxanthines with effects on platelet activation, but not on theobromine. Previous study with theophylline showed an increase of intracellular levels of cAMP, inhibition of ADP release and lactate production in washed human platelet [10]. Therefore, it is suggested that the antiplatelet effects of theobromine possibly is due to the inhibition of platelet phosphodiesterase that prevent the conversion of cyclic AMP to AMP. Agarwal *et al.* (1994) suggested that methylxanthines is an adenosine receptor antagonists. Nevertheless they have reported that addition of theophylline or caffeine to human PRP with replenished adenosine increased the platelet activating factor-induced platelet aggregation. This study also demonstrated the PAF EC_{50} values in patients receiving chronic treatment of theophylline were significantly lowers than in the control subjects [11].

Previous *in vivo* study showed that theobromine significantly reduces total serum cholesterol, LDL-cholesterol and triglycerides, but elevated significantly HDL-cholesterol [12]. Theobromine and caffeine exert stimulant effects of central nervous system by means of adenosine receptor antagonism that results in psychopharmacological effects such as improvement of mood and cognitive functions [6].

Our study showed that theobromine inhibited ADP-induced platelet function in concentration-dependent manner. Choi (2003) shown that *in vitro* treatment of PRP with caffeine reduces the platelet aggregation induced by adrenaline and ADP, but is nonresponsive against collagen and ristocetin platelet stimulation. Interestingly, in some subjects who are responded to caffeine, there was loss of secondary wave after ADP-induced suggesting caffeine impaired the release of endogenous ADP from platelets [13]. Consumption of caffeine and clopidogrel exhibited a potential effect in healthy volunteers and coronary artery disease patients [14]. These effects were seen with the lower ADP-induced platelet aggregation, decrease of platelet surface expression of P-selectin and GPIIb-IIIa receptors and lower of vasodilator-stimulated phosphoprotein (VASP) phosphorylation.

The same study also determined the effects of combination of caffeine and clopidogrel in patients with CAD and observed the decrease of P-selectin and PAC-1 binding but no significant effect on aggregation [14]. Based on these evidence, it could be suggested that theobromine is a platelet inhibitor depending on variations caused by different platelet agonists, and this property may be caused by blocking the adenosine receptor, possibly A_{2A} receptor thus elevate platelet inhibitory cAMP levels.

Previous study has reported the inhibition of ADP and adrenaline-induced platelet activation marker expression of the granular membrane protein CD62P following acute consumption of cocoa beverage in healthy volunteers [15]. Fibrinogen binding with glycoprotein IIb-IIIa involves in platelet activation, but can be inhibited by nitric oxide which increased level of cGMP thus preventing agonist-mediated platelet calcium influx. Cocoa beverage inhibited platelet activation by decreased fibrinogen-binding conformation of platelet glycoprotein IIb-IIIa (PAC1-positive platelets) and inhibited the formation of platelet microparticles [15]. Furthermore, platelet-related primary hemostasis were delayed as measured by prolong closure time in collagen-epinephrine cartridge, but no changes in the collagen-ADP induced closure time after consumption of cocoa beverage [15].

From previous studies, it was suggested that dark chocolate will reduced risk and mortality from cardiovascular disease and demonstrate aspirin-like effects. Consumption of flavonols –rich cocoa decreased the platelet expression of GPIIb-IIIa and P-selectin, with an additive effects when use in combination with aspirin [16]. Moreover, cocoa consumption also prolongs platelet-dependent primary hemostasis in both CEPI and CADP closure time whilst aspirin only decreased CADP-induced closure time [16].

It is noteworthy that nitric oxide inhibits platelet function by increases level of cAMP, thus stimulation of nitric oxide synthase by dark chocolate attenuates platelet function. The beneficial vascular effects of dark chocolate have been extended in healthy smokers known to exhibit endothelial and platelet dysfunction, thus increase risk of atherothrombosis. These effects were demonstrated by increasing flow-mediated dilatation measured by ultrasonography of the brachial artery after 2 hours of dark chocolate ingestion.

5. Conclusion

For the first time, we had demonstrated clearly that theobromine has concentration-dependent antiplatelet effects stimulated by various agonists. The results allows better understanding of the previously reported cocoa beneficial effects to reduce the risk of coronary artery disease as platelet plays a key role in the formation of thrombosis. Further studies to find the exact mechanism of antiplatelet effects of theobromine may corroborate these findings.

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Characterization of Functional Properties of Oxidized Tapioca and Sodium Alginate

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Abstract - The objective of this work was finding possibilities of oxidized tapioca as an alginate substitution. Tapioca was oxidized under controlled temperature and pH with 1.5% oxidants. The result showed that hydrogen peroxide favored the formation of carboxyl group during oxidation, therefore carboxyl content of oxidized tapioca was slight higher than its Na-alginate. Interesting result was found on syneresis characteristic which both oxidized tapioca and alginate have no water release by observing during cooling storage. As a comparison, Na-alginate had significant difference in viscosity, it was ten times more viscous than oxidized starch. Water and oil holding capacity of alginate was higher than oxidized starch. The swelling and solubility capacity determined in temperature 60, 75, and 95 ^{O}C and the results showed values of oxidized tapioca are not far different from Na-alginate. However, oxidation has important role in color that proven by high transparency of oxidized tapioca compare to alginate transparency.

Keywords: functional properties, oxidized tapioca, sodium alginate

1. Introduction

Encapsulation as entrapped of sensitive compound by polymer protected. Encapsulated compound will be protected from the damaged environment conditions, and protected from harmful reactions [13]. Encapsulated can be conducted by many methods, for example is coacervation. The coacervation as encapsulating technique by emulsions forming. Commonly, the encapsulant compound, is anionic polymer and can be matrix forming by calcium ions.

Alginate is widely utilized for encapsulation because it as anionic polymer, can be forms of gel (by egg box formation), bead, microparticle and nanoparticle [4]. Nevertheless, alginate have low transparency and expensive.

Tapioca has possibilities as alginate substitution, because tapioca can be forms of transparency gel, and more cheaper than alginate. Nevertheless, tapioca needs of modification to obtain of anionic properties like alginate. Tapioca modification can be conducted by hydrogen peroxide as oxidizing agent to produce carboxyl groups which anionic charge [9], [12].

Tapioca oxidizing by hydrogen peroxide causes of reaction between oxidizing agent with free hydroxyl groups present in the starch chains to forming of carbonyl and carboxyl groups. This reaction, followed by the hydrolysis of glycosidic bonds, and take place of depolymerisation, so the viscosity would be decrease and the transparency was increase [6]; [11].

Oxidized tapioca possibility as alginate substitution as encapsulant, because it has carboxyl groups as oxidizing produce. This characteristics like alginate, so oxidized tapioca can be forms matrix with calcium ions and can be used as encapsulant by coacervation technique. The

Oxidized maize and oxidized of amaranth starch have been used as encapsulant by spray drying technique [14]. The oxidizing of starch it produce the starch pastes have increased clarity, higher stability and good film properties [6].

The potentially of oxidized tapioca as alginate susbtitute on encapsulated by coacervation technique, not know yet. So need of the research to oxidized tapioca characterization, primary the functional properties and compare to alginate.

2. Material and Method

Tapioca (produce by 99, Malang, Indonesia), used for this experiments. Tapioca 42 grams solved in 100 cc aquadest. This suspension stirred by magnetic stirrer, and added of NaOH 2 N to obtain neutral pH (pH was 7). Stirred by magnetic stirrer 15 minutes, added 1.5 % (v/v) H_20_2 and stirred for 60 minutes. The suspension was sentrifuged for 15 minutes. The solid was dried at 50 ^{o}C for 18 hours. The dried solid was grinded and screened with 80 mesh of sieves, and yield of oxidized tapioca.

The parameters of observations were physicochemical and functional properties of oxidized tapioca and compare to alginate. As control was observe of physicochemical and functional properties of native tapioca. The physicochemical properties were clarity (by absorbance determine at 645 nm) [3], moisture content (thermogravimetric method,

moisture content = weight of water evaporated divide weight of sample) [15], pH (pH of fluid fraction of 10% suspension) [5], carboxyl group content (titration modification) [5], hydrogen peroxide residual [1]. The functional properties were viscosity [2], syneresis (syneresis = weight of water released divide of weight of swollen granules) [3], WHC (WHC = weight of water absorption per gram of sample) and OHC (OHC = weight of oil absorption per gram of sample) [8], swelling power (swelling power = weight of swollen granules divide weight of sample minus weight of soluble starch) and solubility (solubility = weight of soluble starch divide weight of sample) [7], [16].

3. Results and Discussion

3.1. The Physicochemical Properties

The physicochemical properties of native tapioca, oxidized tapioca and sodium alginate is presented in table 1.

Table 1. The physicochemical properties of native tapioca, oxidized tapioca and sodium alginate

Characteristics	Native Tapioca	Oxidized Tapioca	Alginate
Clarity (abs)	0.424	0.033	0.123
Moisture (%)	11.60	9.35	16.15
pH	6.95	7.1	6.27
Carboxyl group (%)	0.116	0.433	0.334
H_2O_2 residual (%)	-	0.672	-

Table 1., showed that the oxidized tapioca had higher transparency (the clarity value was lower) than the native tapioca. Oxidizing tapioca causes hydrolysis of glycosidic bonds, and take place of depolymerisation, so the viscosity would be decrease and the transparency was increase (the clarity value was low) [6]; [11].

The moisture content of oxidized tapioca was lower compare to native tapioca and alginate. The viscosity of oxidized tapioca was lower than native tapioca and alginate, so on drying process more easier and more faster, and produce low moisture content.

The pH of native tapioca, oxidized tapioca and alginate were around neutral. In this way as good characteristic for widely utilized on food processing.

The carboxyl groups content of oxidized tapioca higher than native tapioca. By oxidizing tapioca, would be increase the carboxyl groups content. Some of free hydroxyl groups present in the starch chains are oxidized to carbonyl and carboxyl groups by oxidizing agent [6]. The carboxyl groups content of oxidized tapioca higher than alginate. So the oxidized tapioca was potentially to alginate substitution as encapsulant.

The H_2O_2 residual of oxidized tapioca was 0.672 %. The H_2O_2 residual content of oxidized tapioca was lower than permission maximum concentration by FDA and GRAS, it was 15 %. This condition, showed that the oxidized tapioca was safe used on food.

3.2. The Functional Properties

The functional properties of native tapioca, oxidized tapioca and sodium alginate are presented in table 2., figure 1., 2., 3., 3a., and 4.

Table 2. The functional properties of native tapioca, oxidized tapioca and sodium alginate

Characteristics	Native tapioca	Oxidized tapioca	Alginate
Viscosity (mPa s)			
65 ° C	18.75	12.5	135
47 ° C	57.75	18.0	180
Syneresis (%)	0	0	0
WHC (g water /g starch)	0.89	0.99	8.74
OHC (g oil /g starch)	1.95	0.54	1.54
Swelling power (g/g)			
60 ° C			
75 ° C			
95 ° C	3.63	4.05	11.88
Solubility (%)	7.46	5.18	12.55
60 ° C	9.38	6.05	11.97
75 ° C			
95 ° C	0.0015	1.0	22.6
	0.039	38.4	55.27
	0.125	53.06	74.03

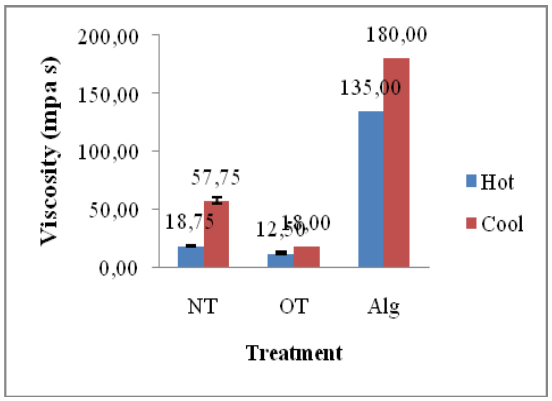

Figure 1. The viscosity of native tapioca, oxidized tapioca, and alginate (NT : native tapioca; OT : oxidized tapioca; Alg : alginate).

Table 2. and figure 1., showed that the viscosity of oxidized tapioca lower than native tapioca. Oxidizing tapioca causes hydrolysis of glycosidic bonds, and take place of depolymerisation, so the viscosity would be decrease [6]; [11].

Table 2., showed that the syneresis characteristic of native tapioca, oxidized tapioca and alginate showed that no water release by observing during cooling storage. In this way as good characteristic for encapsulant.

Table 2. and figure 2., showed that oxidizing tapioca was increasing of WHC but the OHC was decrease. Oxidizing tapioca could be increase the carbonyl and carboxyl groups, so WHC will be increase and OHC will be decrease. Oxidizing starch could be increase the hydrophilic properties [10].

The alginate had the higher WHC than oxidized tapioca. Alginate as an of hydrocolloid which superior binding properties of water [6].

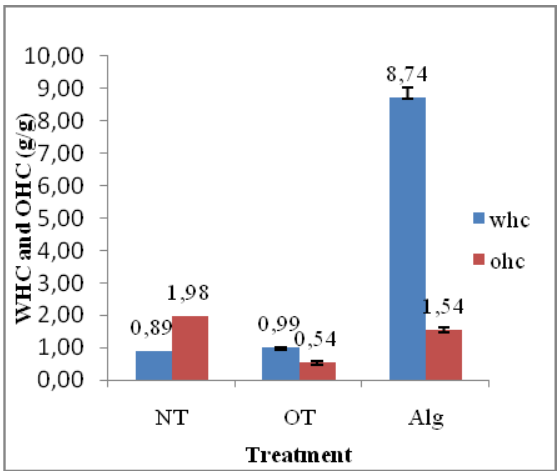

Figure 2. The water and oil holding capacity of native tapioca, oxidized tapioca and alginate (NT: native tapioca; OT: oxidized tapioca; Alg: alginat).

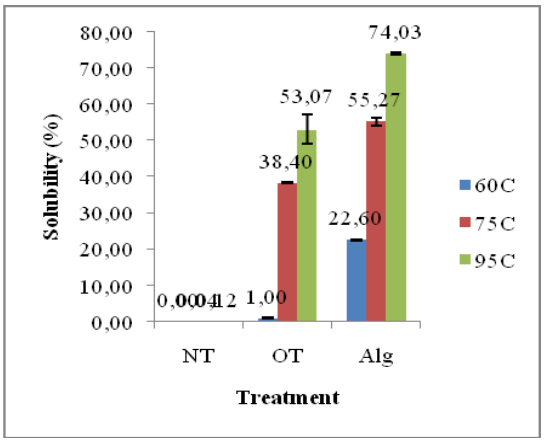

Figure 3. The solubility of native tapioca, oxidized tapioca and alginate.
(NT: native tapioca; OT: oxidized tapioca; Alg: alginat).

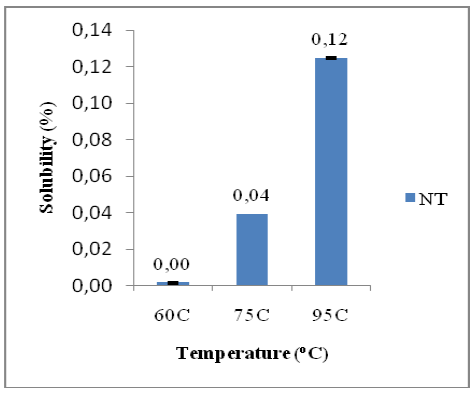

Figure 3 a. The solubility of native tapioca.

From the table 2 and figure 3. it was found that the solubility of oxidized tapioca higher than native tapioca.

Oxidizing tapioca causes some degradation of the polymer chains, and it causes the increasing of carbonyl and carboxyl groups, and hydrophilic properties. So the starch easier to water binding and the solubility would be increase [6], [9].

The table 2., figure 3. and 3a showed that, the increasing of temperature would be increasing the solubility of native tapioca, oxidized tapioca and alginates. The water binding capasity would be increase by increasing of temperature and the solubility would be increase too [6], [9].

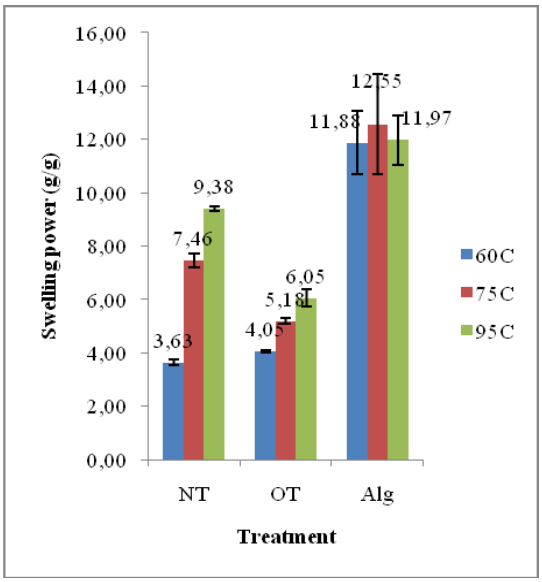

Figure 4. The swelling power of native tapioca, oxidized tapioca and alginate. (NT: native tapioca; OT: oxidized tapioca; Alg: alginat).

The swelling power properties had relationship with WHC. Commonly, if the solubility was high so the WHC and swelling power were high too, and vice versa.

4. Summary

This research showed that based on clarity, carboxyl groups content, and the functional properties (viscosity, syneresis, WHC, swelling power and solubility), the oxidized tapioca had potentially as alginate susbtitute on encapsulated by coacervation technique.

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Characteristics of Fish Oil from Patin Fish (*Pangasius djambal*) Extracted By Dry and Wet Rendering Methods

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Abstract - The main aim of this research is to explore the effect of extraction methods, dry and wet rendering, on the quality and profile of fatty acids in patin fish oil extract. Dry rendering method was carried out in a vacuum oven at 70°C for three hours, while wet rendering was performed under reflux at 100° C for five hours. The data shows that the type and the amount of fatty acids from patin fish oil extracted using both extraction methods were relatively similar. Palmitic acid and omega-9 fatty acids were the most dominant saturated and unsaturated fatty acids in these fish oils, respectively. The quality of fish oil obtained by dry rendering method was better than wet rendering. The number of free fatty acid and peroxide value in dry rendering extract was lower, while saponification and iodine number was higher than the wet one.

Keywords: fish oil, patin, wet rendering, dry rendering, fatty acids.

1. Introduction

Fish oil is a nutrient rich on polyunsaturated fatty acids (PUFA) including omega-3 fatty acids. Two of them are docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). These fatty acids are essential fatty acids which are required by human body system, especially for helping the growth process of the brain, the development of the sense of sight, and increasing the baby's immunity. Saturated fatty acid with short to medium carbon chain is an important antimicrobial agent that protects us from harmful microorganisms in the gastrointestinal system.

Generally, kind of fishes act as potential sources of PUFA are sea fishes such as cod, sardine, whale and hake. However, EPA itself is commonly found in freshwater fishes such as and goldfish, carp, fish cork and catfish [1]. One of freshwater fish which is popular to consume in the world is patin fish [2]. Patin jambal (*Pangasius djambal*) is original patin fish from Indonesia which is widespread in Sumatra, Kalimantan and a part of Java [3]. This kind of catfish has high freshness level, white flesh performance, small bones and skin, lack of fish scent [4], low lipid content [5] and low cholesterol level [6]. The general nutrient contents of this catfish are 13.13-68.60% protein, 1.09-5.80% fat, 1.50% carbohydrate, 0.17-5.0% ash and 59.3-75.7% water [7,6,8]. This patin fish oil is a potential source of unsaturated fatty acids especially for omega-3 [9]. Therefore, patin fish is an interesting sample to study about the quality of fish oil.

Some general methods for fish oil extraction are including rendering, mechanical pressing and solvent extraction. The more modern methods as alternatives to extract the fish oil are enzymatic and ultrasound assisted, cold pressing and supercritical fluid extraction [10]. In fact, although rendering method is a conventional method, it is still popular to use today. Rendering is a simple process since it principally applies heating on wet or dry condition to release triglyceride from animal product and underutilized fish species [11]. Moreover, Astawan [12] showed that the percent yield of fish oil obtained from rendering process is relatively higher than the one obtained from filtration method.

Based on the above information, this study is proposed to compare the quality and profile of fatty acids from patin fish oil obtained by wet rendering and dry rendering processes.

2. Materials and Methods

The sample was fillet of body parts of patin catfish (*Pangasius djambal*). Chemicals used in this research were including sodium chloride, ethanol, acetic acid, chloroform, potassium iodide, sodium thiosulphate, hydrochloride acid, phenopthalein indicator, methanol, starch solution, iodine-bromide reagent, and potassium hydroxide.

2.1 Wet Rendering Method

250 g of sample was put in round bottom flask and added with 500 ml of distilled water. The sample was refluxed for 5 hours at 100°C. The oil phase would float on the surface of the water and then it was separated by pipetting (*first extract*). The residue was pressed to release the remaining fish oil in the meat (*second extract*). *First* and *second extracts* were mixed

and purified using separating funnel in the presence of 2.5% sodium chloride. After heating at 50°C, the oil extract was then centrifuged at 7000 rpm for 20 minutes. The supernatant was specified as fish oil from wet rendering method.

2.2 Dry Rendering Method

250 g of sample was placed on a shelf of pan and roasted in vacuum oven at 70°C for 3 hours. The oil would drop on the pan and be mentioned as *first extract*. The hot fish meat was then pressed to obtain the remaining oil (*second extract*). *First* and *second extracts* were mixed and purified using the same procedure as wet rendering method.

2.3 Characteristics of Fish Oil

The oil yield was calculated as dry fish oil after deducting from its water content. Chemical properties of the oil including acid number, saponification value, peroxide value and iodine number of fish oil extracts were determined using standard method [13].

2.4 Analysis of Fatty Acids in Fish Oil Extract

Analysis of fatty acids was carried out using GCMS-QP2010S from Shimadzu with Agilent J&W DB-1 column (height x ID = 30 m x 0.25 mm), helium as the gas carrier, oven column temperature at 80.0°C, injection temperature at 310.00°C, flow control mode 16.5 kPa, total flow of 40.0 mL/min, and column rate 0.50 mL/min. Oven temperature of program rate, temperature, hold time are (-, 80.0°C, 5.00 min) and (10.0, 305.0°C, 32.50 min). MS conditions were ion source temperature of 250.00°C and interface temperature of 305.00°C.

3. Results and Discussion

Rendering is a way of extracting the oil or fat from material by the use of heating. The main principles of this method are disruption and dehydration of the tissue and breakage of the cells [14] by applying heat to allow oil separation. Rendering method is divided based on the extraction condition into wet rendering and dry rendering process.

In this study, fish meat was extracted by wet rendering method through the use of water in a reflux for 5 hours at 100°C. This method is quite similar to aqueous extraction method. Dry rendering method was carried out without the addition of water but in vacuum oven at 70°C for 3 hours. In this process, applying heating on the material will melt out the fat [15]. Moreover, insoluble materials from both rendering methods were pressed to maximize the oil out of the fish meat and centrifuged to separate the oil and remaining proteins. The fish oils were then characterized for acid number, saponification value, peroxide value and iodine number. The composition of fatty acids in both fish oil extracts were also analysed in this research.

3.1 Quality of Fish Oils from Rendering Process

The results show that wet rendering method produces higher yield of fish oil than dry rendering. However, the presence of water and the use of high temperatures on wet rendering method can hydrolyse the oil results in increasing number of free fatty acids. This phenomenon affects the chemical properties of fish oil in terms of acid number and saponification value. Acid number shows the number of free fatty acids produced by hydrolysis of the oil during rendering process. Higher acid number, the lower quality of fish oil is. Saponification value is a measure of the average of chain length of all fatty acids present in fish oil. The long chain fatty acids found in the oil have low saponification value since they have a relatively fewer number of carboxylic functional groups per unit mass of the oil as compared to short chain fatty acids. Due to the presence of free fatty acids, saponification value of fish oil from wet rendering was higher than dry rendering process. Similar to acid number, the higher saponification value indicates the lower quality of fish oil.

Technological processes of fish oil normally influence the nutritional value of lipids in terms of its level of oxidation. Autoxidation may occur on wet rendering process as a cause of heating at 100°C and no use of vacuum condition (open kettle). Free fatty acids formed by hydrolysis reaction in wet rendering are further deteriorate into peroxides and carboxylic compounds which then decompose into odours meat and give rancid smell to the oil. Fish oil with a high degree of unsaturation will also be attacked by free radical involving oxygen to deteriorate the oil and give rise to peroxide value. The smaller number of peroxide value means that fish oil quality is better. The data shows that dry rendering method give smaller peroxide value than the wet one.

This fact is supported by iodine number of fish oil from wet rendering extract which is smaller than the one obtained from dry rendering. Iodine number indicates the degree of unsaturation or the number of double bond present in the length of the chain. The higher iodine number, the lower becomes the melting point.

Based on those characteristics (table 1), dry rendering method produces a better quality of fish oil than wet rendering method.

Table 1: Characteristics of fish oil from wet and dry rendering method.

Characteristic	*Dry Rendering*	*Wet Rendering*
Yield (%)	1.47	1.81
Acid number (mg KOH/g)	2.10	2.70
Saponification value (mg KOH/g)	117.49	127.81
Peroxide value (mek/kg)	3.19	4.28
Iodine number	106.91	99.07

3.2 Composition of Fatty Acids in Fish Oils

Analysis of the type and quantity of fatty acids contained in fish oil extract was performed by GC-MS. The result reveals that fish oils from both methods contain similar composition of fatty acids (table 2). The total quantity of polyunsaturated fatty acids is more than fifty percent of total fatty acids in the fish oil. The most dominant saturated and unsaturated fatty acids in those fish oils are palmitic acid and oleic acid, respectively. Omega-3 fatty acid content in patin jambal fish oil obtained from this study (3.10%) is in the same number with the one extracted by Hastarini [16] (3.15%) using similar extraction method, wet rendering process. Additionally, both fish oil extracts does not found to contain DHA. Nevertheless, EPA which may possess beneficial potential in mental conditions is presence in higher quantity in fish oil from wet rendering extract compare to fish oil from dry one.

Table 2: Fatty acids content in fish oil from wet and dry rendering method

Carbon Chain	*Fatty Acid*	*Wet Rendering*	*Dry Rendering*
		%	*%*
Saturated fatty acids			
C_{14}	Myristic acid	5.38	4.69
C_{16}	Palmitic acid	30.41	30.65
C_{17}	Margaric acid	0.3	0.33
C_{18}	Stearic acid	8.07	8.45
C_{20}	Arachidic acid	0.19	-
Total of saturated fatty acids		44.35	44.12
Unsaturated fatty acids			
$C_{16:1}\Delta^{9}$	Palmitoleic acid	2.87	2.69
Omega 9			
$C_{18}\Delta^{9}$ ω9	Oleic acid	31.17	32.34
$C_{20:1}\Delta^{11}$ ω9	Gondoic acid	1.96	1.00
$C_{22:1}\Delta^{13}$ ω9	Erucic acid	0.46	-
	Total of omega 9	33.59	33.34
Omega 6			
$C_{18:2}\Delta^{9,12}$ ω6	Linoleic acid	11.69	11.90
$C_{20:4}$ $\Delta^{5,8,11,14}$ ω6	Asam arakidonat	-	0.86
	Total of omega 6	11.69	12.76
Omega 3			
$C_{18:3}\Delta^{9,12,15}$ ω3	α-Linoleic acid	0.64	0.07
$C_{20:5}$ $\Delta^{5,8,11,14,17}$ ω3	Eicosapentenoic acid	2.46	1.52
	Total of omega 3	3.1	1.59
Total of unsaturated fatty acids		**51.25**	**50.38**

According to the data, quality of fish oil obtained from this wet and dry rendering method has fulfilled the standard of International Association of Fish Meal Manufacturers [17].

4. Conclusion

Fish oil quality, in terms of acid number, saponification value, peroxide value, and iodine number, from dry rendering extract is better than from wet rendering extract. However, the profile of fatty acids content from both methods is relatively similar. It was found that palmitic acid and oleic acids are the most dominant saturated and unsaturated fatty acids in both extracts, respectively. In addition, omega three and omega six fatty acids are found in a big portion in fish oils from wet and dry rendering method.

5. Acknowledgement

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Inculcating medical professionalism of medical undergraduates: The IIUM Experience

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Abstract - Nurturing medical professionals who have the necessary expertise to practice safe medicine while at the same time, are committed Muslim is the main learning objective of medical education at the Kulliyah of Medicine, International Islamic University Malaysia since its inception in 1997[1]. This is stipulated in its blueprint for personal and professional development (PPD), known as the Islamic Input in Medical Program (IIMP). Right from the outset, students are reminded about their responsibility to develop the four main attributes of Islamic medical professionalism, summarized as *2E's and 2C's of professionalism* before they graduate and continue it throughout their working life. These are desirable characters of an able and safe doctor sought by patients. The curriculum provides several learning experiences as means to inculcate professionalism.

1. Introduction

Professionalism is defined as "a body of qualities or features as competence, skills, behavior, characteristic, etcetera of a profession". It is the outward visible expression of values, behavior and attitudes that are developed over a period of training. It is the core competency expected of doctors that need to be developed during medical training. One needs to have the necessary expertise to perform one's duty as a doctor, which should be delivered in the most ethical manner. This requires, among others, good communication skill and portrayal of compassion during the period of patient care. This represents the operational definition of professionalism, which evolves around acquiring four main attributes, summarized as 2E's and 2C's. The 2E's stands for Expert and Ethical while the 2C's represent Communication and Compassion.

According to this definition, a doctor is duty bound to discharge his responsibilities towards his patients according to his level of expertise, (E for expertise) and in the most ethical manner (E for ethical). In Islam, one cannot be ethical without having good *akhlaq* as ethic is part of *akhlaq,* which describes the relationship between man and man, man and his creator, i.e. Allah as well as between man and his environment. The other two attributes of medical professionalism that doctors must develop over the years of training and beyond are communication with compassion (the 2C's), which actually means the ability to communicate effectively and compassionately.

This is not an easy task but with dedication and perseverance, one could certainly achieve it. From the perspective of Islam, failure to fulfill this minimum obligation is a sin, since justice could not be upheld and patients are at risk of harm. For a patient, being seen and cared by a knowledgeable and competent physician is all what he wants and is anxious to know whether his ailment is treatable or otherwise, for he wants to return back to his pre-morbid state as soon as possible.

2. Inculcating professionalism

Medical teachers are responsible to nurture their students to achieve a certain level of professionalism, which has to be clearly defined from the outset. Their attitude and practice of professionalism have important impact on the minds of these future doctors. They should motivate these students to become committed to acquire those four main attributes of professionalism mentioned above, which are acquiring expertise expected of a doctor, delivering care to patients in the most ethical manner and ensuring that this is done through good communication skills and portraying compassion. The quest for professionalism should be regularly reminded as one of the learning objectives and enforced in every learning and teaching activities.

Teaching professionalism requires various approaches. Lectures, student workshop, interaction with patients, reflection on real-life practices of healthcare professionals, small group discussions of

"Case Studies", observation and emulating others, especially the teachers and other healthcare professionals, discussion of patient care during bedside teaching and in case reports are among the methodologies used to provide experience and strengthens the commitment to professionalism.

2.1 Medical teachers as role models

Medical teachers, as implementers of the curriculum should be equipped with the knowledge and skills of teaching as well trained to become role models for students. Their professionalism and etiquette *('adab)* in dealing with the patients is a source of inspiration for students to emulate. Role modeling is a powerful force in teaching medical professionalism and is critical to the quality of medical practice and education and to professional self regulation. Marinker used the term *"the hidden curriculum"* to describe the effect of the professional attitudes and behavior of clinical teachers on students and doctors in training. The everyday behavior of clinical teachers is the living demonstration of their expertise, ethics, and commitment to professionalism. What they do and how they do it matters as much as what they say-as in, for example, communicating with patients, students and colleagues; recognizing the limits of their own practice; using clinical audit to improve their practice; applying formative peer appraisal for their own professional development; handling personal criticism; tackling poor performance in themselves and others; and caring for colleagues in difficulty. This is the commitment that should be developed in order to achieve the learning objective of nurturing safe doctors, which is required to safeguard the wellbeing of a community.

2.2 Workshop on Medical Professionalism

A specifically designed workshop on medical professionalism and proper physician etiquette is carried out during the third year prior to the commencement of clinical rotation in the ward. It is a student-centred approach of learning that is integrated with Islamic values, in line with the vision and mission of the University. Its main objective is to provide understanding of the importance of professionalism in patient care and to enhance it further as they undergo the clinical postings until the end of the medical course. Students are divided into small group of ten, where each group is assigned a Case Study to discuss on the issue at hand, that is professionalism. Through active deliberation of these cases, which portray bad professionalism, they would learn the importance of exercising good professionalism and proper etiquette while dealing with patients.

In order to ensure that the learning objectives are reached, each group is assigned a lecturer who shall guide and motivate the students under their charge. More importantly, the teachers should remind students that it is not an option but an obligation to become good doctors, which from the Islamic perspective, is a trust or *amanat* that must be fulfilled. They should be committed to acquiring the four main attributes of professionalism and observing the teachings of Islam in their journey to become good Muslim Doctors.

Each group is then required to demonstrate their understanding on good professionalism and proper etiquette in managing their 'patient' (Case Study) using audio-visual aid, power point and other methods such as role-play. They are also required to write a report and submit it to the coordinator/facilitator of the workshop on the workshop day for grading purposes. Clinical situations (the Case Studies), appropriate for their level, are selected for discussions at this workshop. The examples of case studies are as follows:

i. The first impression – appearance and composure
ii. Examining patient of different gender
iii. Medical confidentiality
iv. Taking verbal consent
v. Refusal of treatment and at own risk discharge
vi. Breaking bad news
vii. Making do'a before a procedure
viii. Solat for the sick

Equally important is that, each group is required to integrate Islamic values while deliberating on the Case Studies and hopefully this skill would be translated into real life experience.

3. Enhancing professionalism

The experience obtained during the workshop should strengthen the commitment of students to improving and enhancing professionalism as they go through their clinical training where they learn to interact with patients and develop the skills of history taking, formulating diagnosis and examining a patient as a professional doctor. Putting into practice the knowledge learnt about medical professionalism, and internalizing it in real-life is not an easy task, but with perseverance and patience, one can achieve it.

An important learning experience that enhances understanding and strengthens commitment to professionalism is the reflective session, *"A reflection on observation of professionalism in the labor room"*, as part of the "Workshop on Obstetric Analgesia" during the Obstetric posting. This learning activity involves every student, either as 'student expert' or as 'active participant'.

There are five main topics discussed, with each topic assigned to two students, who perform the role as 'Student Experts'. The five main topics are:

i. Taking consent
ii. Common drugs in obstetric practice
iii. Monitoring of analgesia
iv. Spinal anesthesia
v. Epidural anesthesia

Two students are assigned to each of the above topics where they are tasked to be the 'expert' who will 'teach' the other fellow friends (i.e. the active participants) during the workshop day. These 'experts' are required to collaborate with the manager of the Clinical Skills Laboratory in setting up the five learning stations with the appropriate learning materials, such as spinal and epidural needles, syringes, drugs, vital signs monitors, consent form, etcetera. Altogether, ten students volunteered to become the 'expert' during the learning session, where they are required to meet the supervisor at least a day prior to the workshop, to rehearse their role and/or clarify doubltful facts about their topic. This exclusive session is also an opportunity to learn other topics prepared by the other 'student experts'. The remaining students (i.e the active participants) are tasked to write one-page report of 200 words on *"Reflection of Professionalism"*, which will be discussed on the workshop day. It is a group activity of at least two persons, where they are required to write on their observations and reflection of professionalism among healthcare professioanls and medical students in the labour room.

On the workshop day, the 'active participnats' are divided into five groups where they rotate to spend fifteen minutes at each station to learn from the 'experts'. The 'student experts' are the resource persons who demonstrate and teach 'the active participants' the assigned topic, for a total of five times. Once the five rotations have been completed, the whole group would then give their feedbacks and comments about the learning experience either as 'student experts' and 'active participants'. The active participants are required to share their reflections on professionalism with their peers. The supervisor give feedbacks, comments and recommendations to these reflections for students to emulate or avoid, with regards to professionalism.

4. Summary

Professionalism is the core competency of doctors that must be developed during medical training. Experiences such as workshop on professionalism prior to commencing clinical rotation and reflective session on professionalism such as during obstetric posting are important activities for students to understand the concept of medical professionalism and proper physician etiquette. These are important platform for developing and strengthening commitment to professionalism in the minds of these future doctors. It is also an opportunity to integrate Islamic values and practicing Islamic culture while dealing with patients.

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Toxicity of Avocado Seed Extract Granules (*Persea americana* Mill.) against The Mortality of *Aedes aegypti* L. Larvae

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Abstract - *Aedes aegypti* L. is a vector that causing Dengue Hemorrhagic Fever. Most of people used synthetic chemical insecticides which have negative impacts, in controlling the dengue vector. Avocado is the one of plants that potential as alternative to the synthetic chemical substances that was commonly used (as botanical insecticide). Seeds of *Persea americana* Mill. were rich in saponins. The present study had the aim of testing the avocado seed extract granules, in order to determine their toxicity (LC_{50}) and LT_{50} during 24 hours towards *Aedes aegypti* L. Concentration serials of granules that used in this study were 50, 60, 70, 80, 90, and 100 ppm for three replications. Each treatment used 20 larvae in the last of third instar to early forth instar. In toxicity tests against *Aedes aegypti* L. larvae, the LC_{50} results obtained was 37,89 ppm, whereas the LT_{50} of 50, 60, 70, 80, 90, and 100 ppm of avocado seed extract granules in series were 20,37; 16,90; 14,12; 14,09; 12,92; and 12,34 hours.

1. Introduction

Aedes aegypti L. is a major cause of disease vectors of dengue in Indonesia [1]. Efforts to control Dengue Hemorrhagic Fever using vaccine virus dengue that has been developed nowadays still have problem to be success [2], so overcome rely heavily on vector control programs [3]. Vector control is still focused on the use of synthetic chemical insecticides, although it leaved a negative impact [4]. Efforts to reduce the negative impacts caused by synthetic insecticides is necessary, to find an alternative insecticides. The one potential alternative insecticide to control the vector is botanical insecticide [5]. There are over 1000 spp. plants that has an ability as insecticides [6]. One of the plants that potential as an insecticide is avocado (*Persea americana* Mill.). Saponins was the highest phytochemical compounds in the avocado seeds [7].

The pesticidal activity of saponins has long been reported. Plant extracts containing a high percentage of saponins was commonly used in Africa to treat watersupplies and wells contaminated with disease vectors; after treatment, the water was safe for human drinking [8]. Activity of saponin was blocking an enzyme in the digestive tract, which would lower the digestibility of certain nutrients (interfere in absorption of food) [9]. Several studies have shown that avocado seeds was toxic. LC_{50} values obtained by testing the toxicity of fresh and dried avocado seeds against *Artemia salina* Leach, were 34,302 mg/L to 42,270 mg/L.[10]. Avocado seed extract was also reported to have an ability as larvicides against *Aedes aegypti* L. with LC_{50} of 16,7 mg/L for its hexane extract [11].

Further research regarding the larvicidal activities of avocado seed extract against *Aedes aegypti* L. according to researchers need to continue because the avocado seed extract had two weaknesses. First, the extract can change the water color when applied directly. Secondly, the storage process of extract was not resistant to the effects of the air. It was necessary to arrange a way to overcome these problems. One was processed extracts into granules. Granules are clumps of particles smaller and is the basis of pharmaceutical preparations which can be further processed into finished pharmaceutical that more stable so it can be stored for long periods [12].

However, this research has differences with previous studies which lies in the material to be tested. Previous studies only examined the avocado seed extract against research object, whereas this research processed the active compounds contained in the avocado seed extract with maltodextrin as a filler, in order to obtain granules. Granules more resistant to the air and not contaminate the water. This solution is an answer of the government allurement to leave the abatesation era. Research on the toxicity of avocado seed extract granules against *Aedes aegypti* L. mosquito larvae that applied on field scale as well as abate powder have not been done, so the researchers wanted to do a study with the title "Toxicity of Avocado Seed Extract Granules (*Persea americana* Mill.) against The Mortality of *Aedes aegypti* L. Larvae ".

2. Research Methods

This experimental study used a completely randomized design. Serial concentrations of avocado seed extract granules used were 50, 60, 70, 80, 90, and 100 ppm in triplicate. The larvae of *Aedes aegypti* L. used in this study were 480 larvae. Each treatment used 20 larvae in 100 ml of avocado seed extract granules. The sample used, was larvae of *Aedes aegypti* L. the last of third instar to early forth instar, with healthy and active movements that results from hatched egg obtained from the Tropical Disease Diagnostic Center, Airlangga University Surabaya, whereas the avocado seeds used in this study were the long red variant avocado seeds obtained from Kalibaru, Kajarharjo, Banyuwangi.

Avocado seed extract granules obtained from mixing 1 g of avocado seed extract and 5 g of maltodextrin as a filler ingredients. Mortality of the larvae was recorded at 24 hours after exposure. Besides of mortality, the observed parameters were temperature (water and laboratory) and humidity of the laboratory in triplicate at 6, 12, 18, and 24 hours. The LC_{50} and LT_{50} values at 24 hours after exposure were analyzed via Minitab 14 for probit analysis.

3. Results

3.1. Avocado Seed Extract Granules and The Toxic Compound

Preparation of granules only needs a gram of avocado seed extract, then mixed with 5 grams of maltodextrin. Granules mass obtained from the mixing process is 5,67 g. Thin Layer Chromatography (TLC) obtained test results that the avocado seed contains saponins of secondary metabolites.

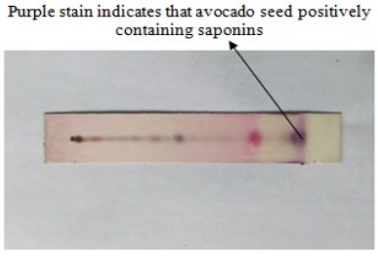

Figure 1: Test Results of Thin Layer Chromatography (TLC) of Avocado Seed Extract at 0,46x Magnification (Source: Personal Collection)

3.2 Final Test Results

Table 1: Mortality of *Aedes aegypti* L. Larvae after 24 Hours Exposure

Concentrations (ppm)	Total Larvae	Mortality of *Aedes aegypti* L. (%)			
		Replication			Mean ± SD
		1	2	3	
K-	20	0	0	0	0 ± 0,00
K+	20	100	100	100	100 ± 0,00
GE 50	20	65	45	55	55 ± 10,00
GE 60	20	70	75	70	71,67 ± 2,89
GE 70	20	75	80	75	76,67 ± 2,89
GE 80	20	75	80	80	78,33 ± 2,89
GE 90	20	85	80	80	81,67 ± 2,89
GE 100	20	85	85	80	83,33 ± 2,89

Specification:
K- : negative control with distilled water (0 ppm)
K+: positive control with 50 ppm abate
GE: avocado seed extract granules

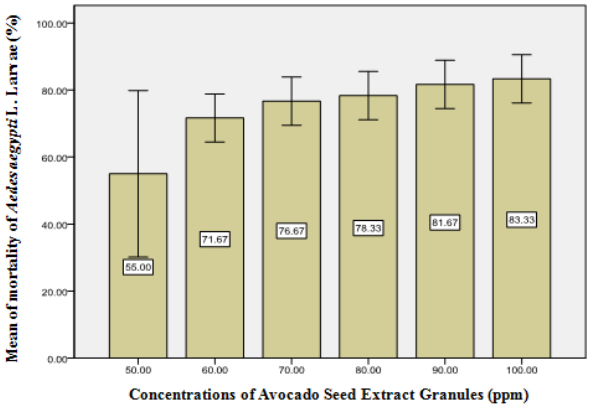

Figure 2: Graph of Relation between Means of Mortality (%) of *Aedes aegypti* L. Larvae and Avocado Seed Extract Granules Concentration (*Persea americana* Mill.) after 24 Hours Exposure

3.3 Data Analysis

Table 2: Results of Probit Analysis LC_{50} after 24 Hours Exposure

Mortality of *Aedes aegypti* L. Larvae (%)	Concentration of Avocado Seed Extract Granules (ppm)	Lower	Upper
50	37,89	17,52	48,76

Table 3: Comparison of Probit Analysis Results between LT_{50} of Positive Control (Abate 50 ppm) and LT_{50} of Avocado Seed Extract Granules at 50 ppm, 60 ppm, 70 ppm, 80 ppm, 90 ppm, and 100 ppm in the 24-Hours Exposure

Concentration of Avocado Seed Extract Granules (ppm)	LT_{50} (hours)	Lower	Upper
Abate 50	3,25	2,82	3,62
50	20,37	17,94	24,06
60	16,90	14,79	19,82
70	14,12	12,73	15,65
80	14,09	12,79	15,48
90	12,92	11,66	14,19
100	12,34	10,97	13,75

4. Discussion

The present study had the aim of testing the toxicity of avocado seed extract granules against the mortality of *Aedes aegypti* L. larvae. Based on the results and analysis of the data showed that the mortality of larvae got higher, with the increasing in concentration of avocado seed extract granules used in the treatment. This is due to the content of toxin contained in the avocado seed extract granules. If the concentration got higher, the active substances that entered the body also became higher, so that the larval resistance against solute decreases and became more susceptible, the result would be a higher mortality. Similarly, exposure to the longer time will cause higher mortality of larvae [13]. This happened because of the toxicity of an insecticide determined by two factors, namely dosage and duration (long exposure). In toxicity tests against *Aedes aegypti* L. larvae, the LC_{50} results obtained was 37.89 ppm in 24-hour long exposure.

Avocado seeds can be referred as an botanical insecticidebecause it proved to be toxic to larvae. TLC test results showed that avocado seed contains saponins which indicated the presence of purple stains because of positive reaction towards sulfuric acid aniseldehida. This color used as a qualitative identification of saponins. Death (mortality) of *Aedes aegypti* L. larvae in this study due to the activity of saponin compounds. Results of probit analysis of toxicity avocado seed extract granules against *Aedes aegypti* L. larvae at 24-hours after exposure (LC_{50}) was 37,89 ppm, while the results of the probit analysis LT_{50} of 50 ppm of avocado seed extract granules was 20,37 hours. LT_{50} of 60 ppm of avocado seed extract granules was 16,90 hours. LT_{50} of avocado seed extract granules of 70 ppm was 14,12 hours. LT_{50} of avocado seed extract granules at 80 ppm was 14,09 hours. LT_{50} of avocado seed extract granules of 90 ppm was 12,92 hours. LT_{50} of avocado seed extract granules of 100 ppm was 12,34 hours.

LC_{50} (Lethal Concentration 50) was concentration of toxin required to cause death in half (50%) of larval populations when entered the body over the specified period of time [15], while the LT_{50} (Lethal Time 50) statistically derived average time interval during which 50% of a given population may be expected to die following acute administration of a chemical or physical agent (radiation) at a given concentration under a defined set of conditions [16]. The body of poisoned larvae turned into a transparent color than healthy larvae (normal) and its body became shrunken. The changing of cuticle was possible due to the degradation of the protein so that left layer of keratin and collagen fibers only. Movement of larvae prior to death was slow, then stop at the base of the cups, and eventually die. Larvae that did not respond to the agitation of the test cups as well as soft stick were counted as dead, while chemical, body-colored transparent when spilled eosin solution, because the cells that make up the body of the larvae was dead so it can not absorb the color [14].

Avocado seed extract granules was a stomach poison to the larvae of *Aedes aegypti* L. because the food contained in the water which the habitat of the larvae, also contaminated by the avocado seed extract granule and can enter the body of larvae when larvae obtained foods. Foods that contained these toxic substances enter into the intestine, then caused a disruption in the *Aedes aegypti* L. larvae mesenteron.

The body of larvae were experiencing paralysis, abdominal color became darker, and the body grew longer than normal larvae. After entered into the digestive tract of insects, toxin across the perithropic membrane and then be bound to a specific receptor on epithelial cell microvilli of midgut (mesenteron) consisting of lipids and proteins, the result disrupted and opened in three-dimensional structure of proteins. It caused the protein denatured, its biological activity broken, and the walls of the digestive tract damaged. Interference with the digestive part of the mesenteron disruption to metabolic processes that result in the production of ATP inhibited and can not repair cells that had been damaged so the larvae eventually death [17].

Saponins was also working as an insect metamorphosis inhibiting toxins. The presence of saponins although in small amounts will bind free sterols in the stomach so that the average amount of sterols taken hemolimfa reduced. Reduction of these sterols can affect insect molting process. Insects were not capable of synthesizing sterol structure by themselves, but they needed for the synthesis of steroids like cholesterol and the insect moulting hormone 20-hydroxyecdysone (20E). That means they must gain them from their food.

Saponins can block sterol uptake. Saponins can form insoluble complexes with sterols, thereby preventing their absorption. If all cholesterol in the food bound to saponins, the insects cannot utilize it. Moreover, the ingested saponins may complex even cholesterol in the body of the insect, and thus suspend the biosynthesis of ecdysteroid. It caused the disturbance of ecdysis (ecdysial failures). Saponins had a steroid and showed structural similarity to ecdysteroid, but few showed principal antagonistic activity on the receptor site for 20 E, i.e. the EcR complex (ecdysteroid reporter) [18].

Saponins was compounds that can decrease the activity of protease enzymes in the digestive tract, thus interfere with the absorption of food. Saponins might be toxic because of their abilities to increase the permeability of the plasma membrane [19]. Saponins can interact with and permeabilize the small intestine mucosal cells of insects, leading to a marked reduction in their ability to transport nutrients [20]. This is suspected to be result of their bipolar structure. The lipophilic component of the saponin could be easily

integrated into the lipid fraction of the plasma membrane [18].

Disorders of the gastrointestinal tract can inhibit the absorption of food. Nutrition deficiencies, as a result of indigestion and assimilation processes, can reduce the rate of growth of insects. This hypothesis supported by a research which have done before. Larvae were tested with saponin lost a lot of weight than the control larvae, just before the pupation [adel]. Saponins can lower the surface tension of the mucous membranes of the digestive tract, so that the walls of digestion tract became broken [22]. In fact, saponins had a specific ability to form porous membranes. This allows the existence of porous membranes become more permeable to other larger molecules [20].

Saponins may interact with the polar heads of membrane phospholipids and the -OH group of cholesterol through OH groups at C_3 or C_{28}, which will result in their later ability to form micelle-like aggregates. Moreover, their hydrophobic aglycone backbone could intercalate into the hydrophobic interior of the bilayer [20]. The amount of glycosides required for permeabilisation was much lower for cholesterol-rich lipid layers than cholesterol-free-membranes [23].

Saponins also worked as a neurotoxin, which had the effect of inhibited the action of kemotripsin, acetylcholinesterase and proteases [24]. Inhibition of acetylcholinesterase enzyme would affect the activity of the muscles in the larvae of *Aedes aegypti* L., because if acetylcholinesterase inhibited by its, the acetylcholine cannot diffuse to the postsynaptic membrane to join with a receptor. When acetylcholine receptor cannot join it, then the depolarization for beginning of the muscle contraction would not be happened. Muscles and nerves were the first systems would damaged, because it lied directly beneath the cuticle [25]. Avocado seed extract granules according to the entry way, also included as a contact poison because it supplied to the water which the habitat of *Aedes aegypti* L. larvae, so that the active compounds (saponins) can enter the larvae body through the particular part of the body surface which had thin cuticle, such as part of the nexus between the segments, the curves was formed from slab body, at the base of the hair and the respiratory tract (spiraculum). The cuticle which exposed to the insecticide will absorbs toxins then carried away by the bloodstream and spread throughout the body. In addition, the cuticle also absorbed residual insecticides [26].

The presence of saponins may cause damage to the cuticle, due to the interaction between the molecules of saponins and the cuticle membrane [27]. The mechanism of saponin was damaged the cuticle of larvae, causing trauma [28]. Symptoms of poisoning caused seed avocado seed extract granules different from the symptoms of poisoning caused by abate. Larvae that died because of abate, the abdomen is more blackened than normal larvae or larvae treated avocado seed extract granule concentration. Abate is an organic phosphate containing phosphorothiate group, which act as anticholinesterase that works to inhibit the cholinesterase enzymes, with the result that causing disruption in neural activity due to the accumulation of acetylcholine at the nerve endings. It caused the death of *Aedes aegypti* L. larvae. Organic phosphate poisoning in larvae followed by restlessness, hyperexcitability, tremors, convulsions, and muscle paralysis (paralysis), the mortality because of the uncapability to took the air for breathing [29]. The observation of larvae in the positive control using abate 50 ppm can be cause of mortality in *Aedes aegypti* L. larvae by 100% within 6 hours. This provides evidence that abate had a very effective killing power as synthetic larvicides compared to botanical insecticide − in this case was the avocado seed extract granules (*Persea americana* Mill.) − despite the negative impact was also less harmful to living things and the environment.

In this study, the observed external factors were temperature and humidity, because other factors were considered homogeneous. Laboratory room temperature during the study ranged from 29 °C to 30 °C. Eggs of *Aedes aegypti* L. were hatched in tap water in growth chamber, at a temperature 30 °C, while the air humidity during the study ranged from 73% to 75%, so it can be concluded that the observed external factors did not really give a great influence on mortality of larvae of *Aedes aegypti* L.

Security level of an active ingredient in the study needed to be reviewed so that not to cause further new negative impact on the environment and ecosystem. Avocado seed extract granules made from a mixture of a gram of avocado seed extract granules and 5 grams of maltodextrin as a filler, safe when applied to the waters. It is based on several previous studies. Excipients in the form of maltodextrin in the manufacture of granules safe to use. Maltodextrin had been widely recognized as nonirritant and nontoxic substance [30].

5. Conclusions

The following conclusions can be drawn based on the experimental results demonstrated in the present study:

1) In toxicity tests against *Aedes aegypti* L. larvae, the LC_{50} results obtained was 37,89 ppm
2) The LT_{50} of 50, 60, 70, 80, 90, and 100 ppm of avocado seed extract granules in series were 20,37; 16,90; 14,12; 14,09; 12,92; and 12,34 hours.

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Solvent Extraction of Lycopene from Tomato (Lycopersicum esculentum Mill.)

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Abstract - Tomato is one of the main sources of lycopene which benefits as good antioxidant. This study has a main aim to optimize the solvent extraction method of lycopene in tomato. Kind of solvent used for the extraction were n-hexane:acetone:methanol in various compositions (1:2:1; 1:1:1; 2:1:1) and petroleum ether (PE):acetone (3:1). The crude extracts were eluted with n-hexane:PE (2:1) in silica column chromatography and resulted in four fractions with different colours. The second fraction was red and showed a visible spectrum pattern and three peaks of maximum wavelength similar to that of lycopene standard. The result shows that n-hexane:acetone: methanol (1:2:1) was the best solvent mixture in facilitating extraction of lycopene from tomato. In addition, this study has confirmed that the intensity of RGB levels on tomato correlated with the content of lycopene.

Keywords: lycopene, tomato, solvent extraction, RGB value, carotenoids.

1. Introduction

Lycopene is an acyclic hydrocarbon with 13 unsaturated double bonds which 11 of it is in linear conjugation. Due to the conjugation effect of double bonds, lycopene is a great antioxidant with the power to catch singlet oxygen is twice that of β-carotene [1,2] and ten times that of α-tocopherol [3].

Utilization of lycopene as a functional food products and nutritional supplements have attracted a lot interests today, especially from food and pharmaceutical industry. In fact, future demand on stock of lycopene grows rapidly, while its current production is small [4,5]. Several synthetic pathways for lycopene have been proposed, but most of them seem to be a high cost production. Alternatively, biotechnological production of lycopene using microorganisms [6,7] and cell culture [8] have been developed, but are still not efficient with a low yield of lycopene. As a result, enhancing the availability of lycopene from natural sources such as tomato is very encouraged. In other words, consideration the better method for extraction, characterization and determination of lycopene is required.

Due to the fact that biosynthetically lycopene is formed through mevalonic acid cycle in the cytosol [9], suitable extraction method to release lycopene which is deeply embedded within chromoplast membrane [10] is required. However, the available solvent extraction technologies do not seem to allow a fast and economic recovery of the carotenoid. Zuorro and Lavecchia [4] also stated that solvent extraction only produces extremely low number of lycopene extract. In addition, Naviglio [11] and Sadler *et al* [12] mentioned that extraction procedure commonly involves a complex purification step and a high use of organic solvents. Hence, some modern technologies to extract lycopene are carbon dioxide supercritical fluid [13,14,15], enzyme aided extraction [4,16,17], and ultrasonocation [18]. However, conventional method based on solvent extraction is popular to use since it is a simple and convenient procedure, inexpensive, and suitable for a large scale process.

Pre-treatment of sample with a polar solvent will preserve lycopene from degradation and prevent from partial collapse of polysaccharide network which could hinder solvent penetration during extraction [4]. Alkaline saponification also helps to release lycopene by removing the glycerides and free fatty acids in sample [8,19]. Therefore, in this study, tomato extract was pre-treated with methanol then followed by organic solvent extraction to obtain lycopene extract.

The solvent chosen for lycopene extraction should efficiently extract all carotenoids present in the sample. Solvent with low boiling point is normally used to avoid prolonged heating in evaporation step, though the use of water as a green alternative solvent has been frequently used [11]. In fact, acetone and PE have been widely used in carotenoid extraction to help penetration of solvent into cells [20].

Solvent mixtures of n-hexane:acetone:ethanol and ethanol:pentane have been studied as solvent for extracting lycopene [15,21-23]. To the best of our knowledge, no study is applying solvent mixture of n-hexane:acetone:methanol to this case. Hence, this study explored the possibility of this solvent mixture in extracting lycopene.

The red intense colour of tomato skin normally indicates the high content of carotenoid pigment inside, especially lycopene. In fact, different colours of tomato skin affect the intensity of red, green and blue factors (RGB). Hence, this study also determines correlation between the colour of tomato and its lycopene content.

2. Materials and Methods

The samples were tomato fruits (*Lycopersicum esculentum Mill.*) obtained from traditional market in Jember. Chemicals used in this study were acetone, methanol, n-hexane and petroleum ether (PE).

2.1 Extraction of Lycopene from Tomato

1 kg of crushed tomatoes was blended and then was put in a beaker glass. Pre-treatment was carried out by adding methanol into tomato juice and stirred for 5 minutes. The mixture was then filtered and put in the same quantities into four erlenmeyers coated with aluminum foil. The extraction solvents were added into each erlenmeyer with a ratio of 5 times volume of tomato slurry. The solvent mixture used were PE:acetone (3:1) and n-hexane:acetone:methanol in the ratio of 2:1:1; 1:2:1; and 1:1:1. All mixtures were extracted in a shaker at 150 rpm for 30 minutes. Then each of them was transferred into separating funnel and added 10 mL of distilled water. Separation was done to form two phases. The upper layer, as organic phase, was separated and subjected to evaporation using a rotary evaporator. The concentrated extract was purified using silica column chromatography in an optimum eluent for carotenoid separation.

The eluents were optimized using thin layer chromatography (TLC) method. Crude extract of lycopene was spotted on silica gel 60 TLC plate and eluted by various eluents including PE:acetone (8:2); PE; n-hexane; n-hexane:PE (2:1). Spots formed on the TLC plate were analyzed under UV light and quantified for their *Rf*.

2.2 Purification of Lycopene Extract

The crude extract of lycopene was passed over silica column chromatography using the optimum eluent of n-hexane:PE (2:1). Fractions in different colors were collected in different vials. Each fraction was scanned for its visible spectrum using UV756CRT spectrophotometer and recorded its absorbance at 470 nm, as it equals to its lycopene content.

2.3 Determination of Relationship between Tomato Colour and Lycopene Content

Three tomatoes in different colours, green; yellow; and red, were washed and recorded for their weights. Those tomatoes were shot using a camera (height from sample was 17 cm vertically) with a black background and the presence of 20 watt light bulbs in a distance of 33 cm and 40 cm high lights. The pictures were then analysed for their RGB values. All tomatoes were subjected to the assay of lycopene content using visible spectrophotometer at 470 nm.

3. Results and Discussion

Lycopene is a secondary metabolite which presents in chromoplast and cytosol from cells of tomato fruit. Solvent extraction method is an appropriate way to extract lycopene. This study aims to optimize the solvent compositions in extracting lycopene from tomato. The crude lycopene extract was then purified using silica column chromatography.

Due to large amounts of water content in tomato sample, the use of a water immiscible organic solvent such as acetone and methanol should be used to allow better solvent penetration. Therefore, in order to increase solvent penetration, tomato juice has been previous soaking for 5 minutes in methanol to soften the cell wall. Prolonged soaking should be avoided to prevent isomerization and degradation of the carotenoids. Pre-treatment of tomato juice with methanol was attributed to the swelling of plant tissue which resulted in less compact to tomato paste matrix and favouring the diffusion of the extraction solvent.

Eluent used for lycopene purification was previously optimized using TLC method in silica plate. Several eluents optimized in this research were including PE:acetone (8:2); PE; n-hexane; and n-hexane:PE (2:1). TLC method with eluent of PE only generated one yellow spot. It is suggested that this solvent could not separate carotenoid compounds in tomato extracts. Each TLC method using eluent of PE:acetone (8:2), n-hexane and n-hexane:PE (2:1) separated the extract into two spots in yellow and orange colours. The first spot (*smaller Rf*) possibly contained xanthofil, while another spot may contain lycopene and β-carotene.

Table 1: Optimization of Eluent for TLC and Column Chromatography

Eluent	*Rf 1*	*Rf 2*
PE-acetone (8:2)	0.340	0.96
PE	0.688	-
n-hexane:PE (2:1)	0.626	0.791
n-hexane	0.215	0.538

Based on Table 1, the result shows that eluent of PE:acetone (8:2) produced the best separation (*Rs* value) compared to others. However, in this case, the second spot (*bigger Rf*, 0.96) was very close to the solvent front. Therefore, eluent of PE:acetone (8:2) was eliminated from the list. Eluent of n-hexane:PE (2:1) produced larger *Rf* values than eluent of n-hexane in separating the compounds. It means that when eluent of n-hexane:PE (2:1) was used in column chromatography, the first compound in the extract would separate and come out quickly. This work could save time and number of eluent used in column chromatrography. Based on data from Table 1, eluent

n-hexane:PE (2:1) is the most suitable eluent for separation of compounds in tomato extract.

Open column chromatography is the classical method for separation and quantitative analysis, while high performance liquid chromatography is the modern one [24]. In this research, separation of carotenoid compounds in tomato extract using silica column chromatography was followed visually based on the colour of each fraction. This separation method resulted in four fractions, namely fraction 1-4 with the colour in order were yellow, red, purple and yellowish green. Those fractions were scanned for their spectrum patterns and maximum wavelengths absorption using visible spectrophotometer (Figure 1). Spectrum pattern for fraction 2 was similar to the spectrum of lycopene standard. In addition, there were three peaks recognized as the maximum wavelengths of lycopene which conformable to other reference [20] at 444 nm, 470 nm, and 502 nm. This fact indicates that fraction 2 absolutely contained lycopene. Meanwhile, fraction 1 and 3 did not match with the spectrum pattern and regarded peaks of lycopene standard.

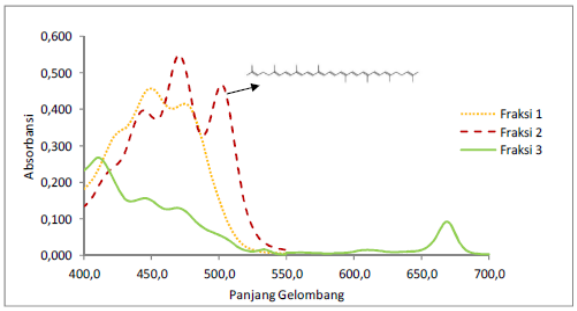

Figure 1: Visible spectrum of fraction 1-3 in PE.

The effect of variation compositions of the solvent for lycopene extraction has been studied. Since carotenoids in solution obey the Beer-Lambert law, thus the lycopene extract was quantified spectro photometrically.

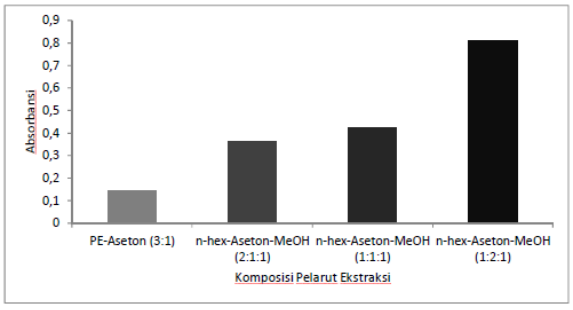

Figure 2: Absorbance of lycopene in various compositions of solvent for extraction

Figure 2 illustrates that n-hexane:acetone: methanol (1:2:1) is the best solvent to facilitate extraction of lycopene from tomato. Following the previous solvent, n-hexane:acetone: methanol in the ratio of 1:1:1 and 2:1:1 also assisted the lycopene extraction, but in lower quantities or absorbance. There is an appreciable difference was observed in lycopene content by increasing the quantity of acetone in the mixture of n-hexane:acetone:methanol as extraction solvent. It can be concluded that the presence of methanol and acetone has an important role on gaining of lycopene in tomato extract. The more quantity of acetone used in solvent mixture, the bigger number of lycopene was obtained. Theoretically, acetone is soluble in methanol and n-hexane. When it is presented in bigger proportion compare to other solvents, acetone will become a solvent bridge so that maximizes the lycopene extraction from polar phase (methanol) into non polar phase (n-hexane).

Chromophore in lycopene, that is eleven conjugated double bonds, is able to absorb light in the visible region. As a consequence, lycopene generates a red colour in tomato skin. In this research, interpretation of tomato colour as the function of lycopene content was studied. The intensity of colour in the photo is normally expressed in RGB format. The RGB value of selected tomato was then correlated with the level of lycopene in tomato fruit.

Table 2: Relationship between the Colour of Tomato Fruit and the Level of Lycopene.

Color of the Fruit	Intensity (RGB value)			Lycopene Level (µg/g)
	Red	Green	Blue	
Green	188.89	243.34	21.44	0.239
Yellow	248.58	249.47	30.46	1.272
Red	249.84	141.62	73.32	13.387

Table 2 shows that the greater intensity of Red in the picture of tomato, then it would be the higher level of lycopene inside was. Green reflectance appeared in bigger intensity for green to yellowish tomatoes, while it appeared in smaller intensity for yellowish to red samples. Therefore, this interpretation method (RGB) can be useful for predicting the content of lycopene in tomato fruit.

3. Conclusion

The optimum composition of the solvent mixture in the extraction of lycopene from tomato was a solvent mixture of n-hexane:acetone:methanol (1:2:1). Both the maximum wavelengths absorbance and the spectrum shape of pure lycopene extract in fraction 2, which was obtained from column chromatography using eluent of n-hexane:PE (2:1), were similar to lycopene standard.

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Synthesis and Characterization Core-shell ZnO/TiO2 as Photoanode Material in Dye Sensitized Solar Cells (DSSC)

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Abstract - Core-shell ZnO/TiO_2 synthesized on indium tin oxide glass and used as fotoanoda in Dye sensitized Solar Cells (DSSC). Synthesis of ZnO core has been conducted by chemical deposition method at 90^{o}C for 6 hours from zinc nitrate and metenamin. TiO_2 shell has synthesized from titanium isopropoxide in sol-gel method. TiO_2 deposition on ZnO rods using spin-coating method. XRD Characterization results indicate that qualitative peak for TiO_2 and ZnO with high crystallinity. Mole ratio of TTIP/water as Hydrolysis ratio parameters (1:2, 1:4, 1:6) resulted in variation of TiO_2 crystal size which is equal to 61.49; 56.71 and 54.30 nm. The annealing temperature increases the crystallinity of ZnO and TiO_2. Morphological characterization using SEM obtained ZnO rods and TiO_2 in micrometer-sized. Basic performance of DSSC can be obtained from V_{oc} values. The highest V_{oc} values of the results fabrication fotoanoda ZnO/TiO_2, carbon and extract of red cabbage is 344.4 mV

1. Introduction

Dye Sensitized solar cells (DSSC) have a good potency to be developed as renewable energy devices in future. Comparing to silicon based cells, DSSC are lower in manufacturing cost but their photoelectric conversion efficiency (PCE) is not high to meet the criterions of industrial applications. However, there are still remaining challenges to improve the efficiency and stability of DSSC [1].

Zinc oxide is one of the most promising functional materials because of its direct band gap of 3.37 eV. ZnO shows good flexibility in synthesis and morphologies of particles. Usually ZnO is used as photoanode material of solar cell. But there are some problem in the dye adsorption procedure. These problems are mainly related to the high acidity of the carboxylic acid binding groups of the dyes that can result to dissolution of ZnO and precipitation of Zn^{2+}/dye complexes [2].

Debility of ZnO can be improved with coating TiO_2 in surface area of ZnO particles. As a good chemical stability semiconductor, TiO_2 is also have much closed direct bandgap (3.2 eV) with ZnO. Combining both of them can create a material of photoanode with high mobility electron and good chemical stability [3].

In this work, we purposed to synthesis core-shell ZnO/TiO_2 as photoanode material of DSSC. Core ZnO has been synthesized by using chemical deposition method to get uniformly rods ZnO and TiO_2 particles that synthesized via sol-gel. Meanwhile the distribution of TiO_2 on the ZnO's surfaces was done by spin coating process. The resulting core-shell ZnO/TiO_2 were characterized by using X Ray Diffraction (XRD) and Scanning Electron Microscopy (SEM). There are two parameters has been observed. They are hydrolysis ratio and annealing temperature. XRD and SEM analysis showed that ZnO/TiO_2 were successfully synthesized in microsize. The photoanode is also fabricated with carbon catalyst and extract of anthocyanin from red cabbage to analyze the voltage open circuit (V_{oc}) as basic performance of DSSC.

2. Experiment

2.1 Synthesis

Core ZnO has been synthesized on the Indium Tin Oxides glass (ITO) via chemical deposition process. First, we prepared solutions of 100 mM of $Zn(NO_3)_2.6H_2O$ and 100 mM hexamethylenetetramine ($C_6H_{12}N_4$) (Aldirch, 99%). ITO glass was immersed in that solutions for 6 hours at 90^{o}C. Shell TiO_2 was synthesized from 0,4 M Titanium isopropoxide ($[TiOCH(CH_3)_2]_4$/TTIP (Aldirch, 97%) in ethanol solution by using sol-gel method. Hydrolysis reaction was promoted by adding water to the solution (acid condition). Amount of water were setting in the different ratio mol TTIP/water (1:2, 1:4, and 1:8). The ITO glass that contained ZnO was dropped by $Ti(OH)_4$ sol and was spinning for twenty seconds at constant rate and the product was annealed at 450^{o}C, 550 ^{o}C and 650 ^{o}C for 1 hour.

2.2 Characterization and cell fabrication

The structural characteristic of the ZnO/TiO_2 were observed by X ray diffraction (XRD) using Cu-K_α radiation and diffraction angles (2θ) 20°-70° (PAN-analytical PW3373). Scanning electron microscopy (SEM, JEOL-JSM-6360LA) was used to analysis characteristic of morphology. Performance of DSSC was observed by multimeter. The cell was arranged by photoanode, dye, electrolyte and carbon catalyst. Sensitizer was extracted from red cabbage in aqueous solution. Core-shell ZnO/TiO_2 were immersed in dye for 8 hours, after that photoanode was droped by electrolyte (NaI/I_2) and arranging with carbon catalyst.

3. Results and discussion

XRD characterization of core-shell ZnO/TiO_2 was successfully sensitized based on the comparing peak of sample diffraction and the standard diffraction of ZnO (*Zink oxide,* JCPDS 80-0075) and TiO_2 (*Titanium oxide,* JCPDS 84-1286).

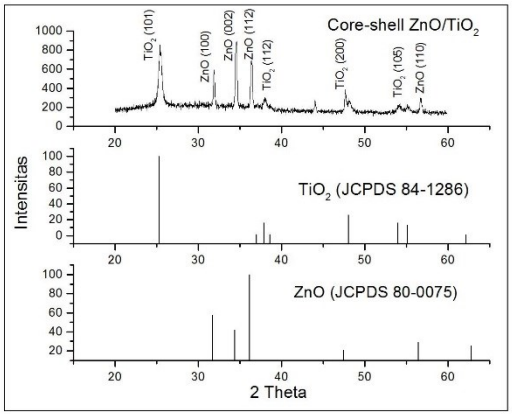

Fig 1. XRD patterns of core-shell ZnO/TiO_2

Fig.1 shows that XRD pattern of sample similar with standard powder diffraction patterns, so the material which was synthesized absolutely ZnO and TiO_2 anatase.

Initial process of synthesis ZnO rods was reaction between $Zn(NO_3)_2$ and metenamine as a weak base. At 90°C, Metenamine will produce OH^- ions to initiate the hydrolysis of zinc nitrate. The formation of zinc oxide consist of two main reaction. That are hydrolysis and dehydration.

$$(CH_2)_6N_4 + 6\,H_2O \rightleftharpoons 6HCHO + 4NH_3 \quad (1)$$

$$NH_3 + H_2O \rightleftharpoons NH_4^+ + OH^- \quad (2)$$

$$Zn^{2+} + 2OH^- \rightleftharpoons Zn(OH)_2 \quad (3)$$

$$Zn(OH)_2 \rightleftharpoons ZnO + H_2O \quad (4)$$

In hydrolysis process (3), Zn^{2+} converted into $Zn(OH)_2$ after reacting with OH^- ion and zinc hydroxide were dehydrated to be an oxide.

TiO_2 particles were synthesized from titanium isopropoxide precursor. There are two main process for sol-gel method

$$Ti(OR)_4 + 4\,H_2O \xrightarrow{H^+} Ti(OH)_4 + 4\,ROH \quad (5)$$

$$\equiv Ti{-}OH + HO{-}Ti\equiv \xrightarrow{\text{yields}} \equiv Ti{-}O{-}Ti\equiv + H_2O \quad (6)$$

Titanium isopropoxide was hydrolyzed under acid condition to form a $Ti(OH)_4$ sol. The second reaction is condensation betweeen two or more molecule of titanium hidroxide to obtain TiO_2 in the end of processes. Controlling the size of particle TiO_2 depended on pH, hydrolysis ratio and annealing temperature [4,5,6].

$Ti(OH)_4$ sol was synthesized in low pH with adding diluted HCl. In acid condition, hydrolysis reaction became more dominan than condensation and resulting the particle in small sized. The $Ti(OH)_4$ sol was deposited in ZnO rods via spin coating method [7].

Spin coating is currently the predominant technique employed to produce uniform thin films of photosensitive organic materials with thickness of the order of micrometers and nanometers. Spin coating can be effectively modeled by dividing the whole process into four stages, that processes consist of deposition, spin up, spin up and evaporation of solvent. Substrate ZnO rods on ITO glass attached to spin coating devices, and then drops by $Ti(OH)_4$ sol. Substrate was spinned for 20 seconds and dried at room temperature. The last process was annealing of core-shell at certain temperature to get a highly cristallity of ZnO/TiO_2.

XRD analysis toward the core-shell ZnO/TiO_2 in different hydrolysis ratio was showed in fig 2.

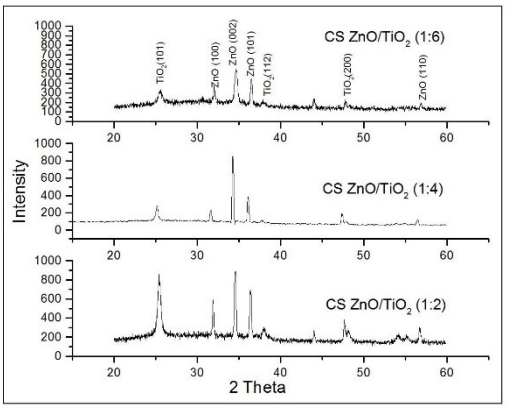

Fig 2. XRD pattern of core-shell ZnO/TiO_2 at 450°C

Fig 2. shows the broadening of diffraction peak especially in 2θ = 25°, widht of diffraction peak influence the particle size. It proved by Scherrer equation :

$$d \approx \frac{K.\lambda}{B.sin\theta}$$

Hydrolysis ratio	angle (2θ)	FWHM (°)	d (nm)
1 : 2	25,57	0,2652	61,49
1 : 4	25,14	0,2871	56,71
1 : 6	25,45	0,3000	54,30

Table 1. FWHM TiO_2

Table 1 shows the relation of hydrolysis ratio, full widht at high maximum (FWHM) and crystal diameter (*d*). Increasing hydrolysis ratio caused decreasing diameter of crystal. The reason is higher mole of water leaded hydrolysis to be more dominant process than condensation. The result is the formation of a lot of crystal nucleus in small size.

Applied of annealing temperatures gave a different XRD pattern for each of hydrolysis ratio. Higher intensity of diffraction peak was obtained in higher temperature of annealing. It is shown in fig 3(a), 3(b) and 3(c).

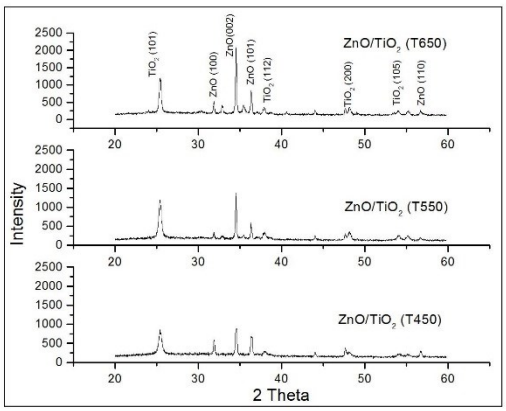

Fig 3 (a). XRD patterns of core-shell ZnO/TiO_2 (ratio 1 : 2)

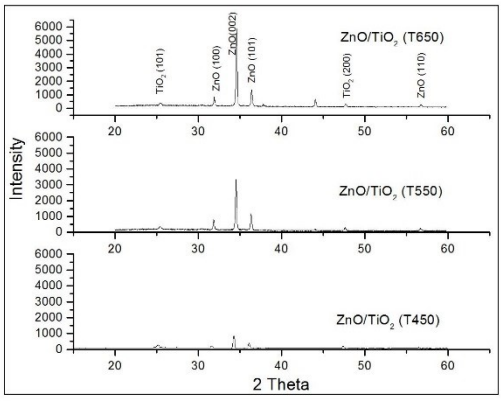

Fig 3 (b). XRD patterns of core-shell ZnO/TiO_2 (ratio 1 : 4)

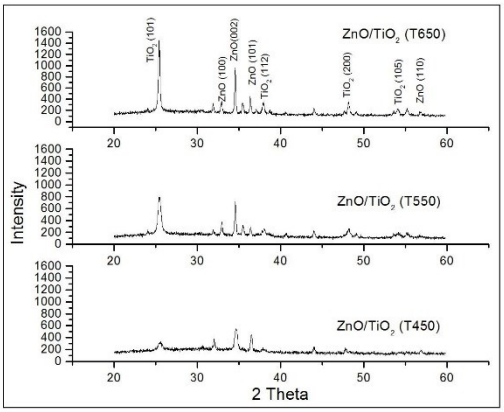

Fig 3 (c). XRD patterns of core-shell ZnO/TiO_2 (ratio 1 : 6)

Temperature of annealing process influence the energy of atoms in crystal and leaded to high crystallity materials. The best anatase structure were obtained at 550°C, it is shown by the high intensity of diffraction peaks of TiO_2 in certain diffraction angles. while the rutile structure were appeared at higher temperature (650°C).

SEM analysis show a different morphology and particle size of crystal for different annealing temperature.

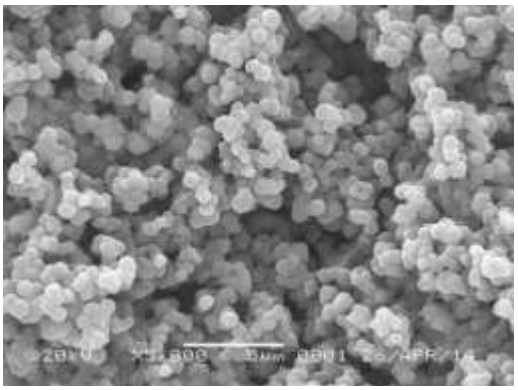

Fig 4 (a). morphology of core-shell ZnO/TiO_2 at 450°C (Rw = 1:2)

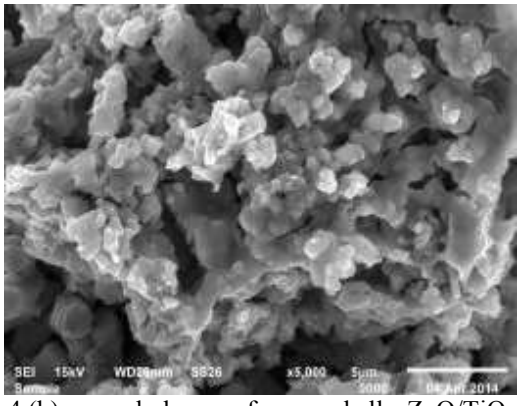

Fig 4 (b). morphology of core-shell ZnO/TiO_2 at 550°C (Rw = 1:2)

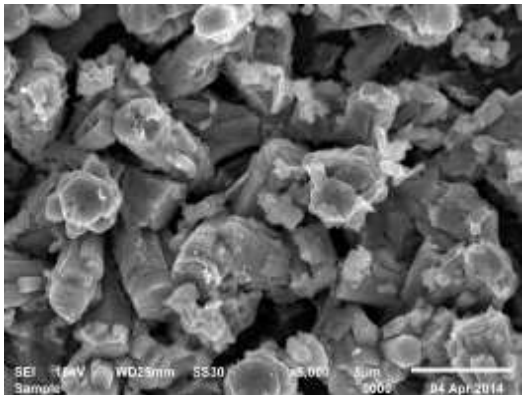

Fig 4 (c). morphology of core-shell ZnO/TiO_2 at 650^oC (Rw = 1:2)

Fig 4 (a), (b) and (c) show the increasing of crystal growth. ZnO crystals easily obtained at higher temperature of annealing, meanwhile the TiO_2 crystals were already exist at lower temperature due to their position in surface of material. SEM analysis were also showed that the synthesized particles of ZnO/TiO_2 in microsize range.

Measuring the voltage open circuit (V_{oc}) of DSSC have been done. Different annealing temperature show different V_{oc} value. It is shown in fig.5

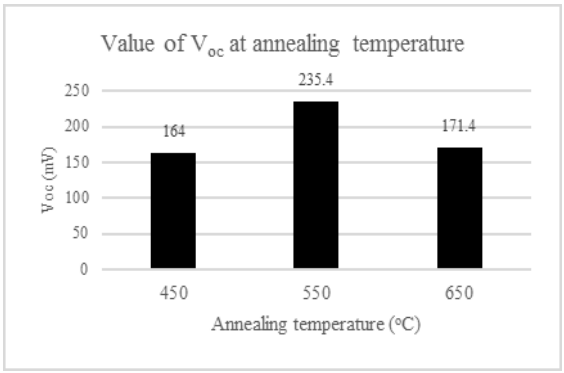

Fig.5 V_{oc} value of core-shell ZnO/TiO_2 (R_w=1:2)

Core-shell ZnO/TiO_2 that were synthesized at 550^oC have highest V_{oc} value (235.4 mV). It was caused they have the best structure of TiO_2 anatase and optimum adsorption of dye.

Variation of hydrolysis ratio was giving different V_{oc} value for each fabricated solar cell. The V_{oc} measurement was doing at a constant annealing temperature (550^oC).

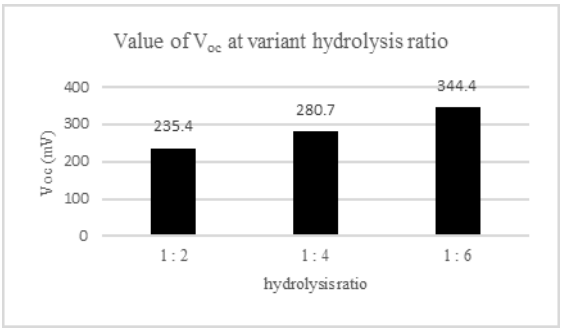

Fig. 6 V_{oc} value of core-shell ZnO/TiO_2 at variant hydrolysis ratio

Fig 6. show the measurement of V_{oc} value for core-shell ZnO/TiO_2 that were synthesized at variant hydrolysis ratio (1:2, 1:4 and 1:6). The result is increasing V_{oc} value in a row of increasing hydrolysis ratio. In 3 repetition of measurements, hydrolysis ratio 1:6 still existed in highest V_{oc} value due to its crystalline size that became smaller than other hydrolysis ratio. The smaller crystal would increase the dye adsorption in surface of material. So it could enhance the basic performance of DSSC especially their V_{oc} value. Overall, the performance of DSSC was not only depended on material of semiconductor, but also depended on dye, electrolyte, catalyst and technic of DSSC fabrication [8].

4. Summary

In summary, XRD and SEM analysis show the core-shell ZnO/TiO_2 were successfully synthesized in microsize. Parameters hydrolysis ratio that is applied to the mole ratio of TTIP / water (1:2, 1:4, 1:6) resulted in the TiO_2 crystal size variation of 61.49 nm, 56.71 nm and 54.3 nm. Increasing the annealing temperature increases the crystallinity of ZnO and TiO_2 that was synthesized. Based peformance of DSSC can be obtained by measuring open circuit voltage (V_{oc}). The higgest V_{oc} is 344.4 mV for fotoanode ZnO/TiO_2 that was fabricated with catalyst carbon and extract of anthocyanin from red cabbage as sensitizer.

5. Acknowledgments

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Antibiofilm Activities of Red Ginger Essential Oil (Zingiber officinale var rubrum) against Staphylococcus epidermidis

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Abstract - Staphylococcus epidermidis is the normal bacterial flora of humans which can cause infection in certain circumstances. This microorganism has emerged as one of the major causative agents of nosocomial infections. Moreover *S. epidermidis* can produce a number of molecules contribute to the formation of biofilms. The existence of biofilm will increase the incidence of antibiotic resistance 10-1000 times from its initial state. This study used a suspension of red ginger essential oil to determine its ability as an antibacterial and antibiofilm. Antibacterial activity was determined using agar diffusion method while biofilm inhibition and degradation activity were determined using the microtiter plate assay. Crystal violet were used to stain the biofilm and optical density (OD) was measured at λ 595 nm. The results showed that the diverse value of suspense red ginger essential oil has antibacterial and antibiofilm activity againts *S. epidermidis* and exhibited different activity.

Keywords : Red ginger essential oils, *S.epidermidis*, antibacterial, antibiofilm.

1. Introduction

In certain circumstances, *S. epidermidis* can cause infection [1]. During two decades, *S. epidermidis* has emerged as a major causative agent of nosocomial infections[2].

Nosocomial infections are becoming a serious problem. Research conducted by Musadad and Lubis (1992) on nosocomial infections in a hospital in Jakarta showed that 13.54% of the 96 patients had nosocomial infections and when the smear test on nurse hands was done, there *E.coli* at 3.2%; *Pseudomonas aureoginosa* 6.4%; and 12.9% of *Staphylococcus sp* [3]. Nosocomial infections also occur in Setjonegoro Wonosobo regency hospital. The incidence of nosocomial infections in hospitals has increased from 2010-2011 (0.37% become 1.48% cases) [4]. Nosocomial infections are also found in the burn patients in RSCM due to *S. epidermidis* and *Klebsiella pneumoniadan* bacteria [5].

In addition to the causes of nosocomial infections, *S. epidermidis* produces some molecules, especially proteins and exopolymer such as exopolysaccharide PIA (*polysaccharide intracellular adhesion*). The PIA exopolysaccharide contributes to form biofilm [6].

The presence of biofilm increases the resistance to antibiotic of 10-1000 times from its original state [7]. Approximately 60% of *S. epidermidis* strains are resistant to nafcillin [8]. Besides, *S. epidermidis* is also resistant to meticilin, aminoglycosides and macrolides, as well as most other antibiotics such as tetracycline, chloramphenicol, and clindamycin [6].

Thus, efforts are needed to control infection and biofilm forming by *S epidermidis*. One example of a well-known medicinal plant in Indonesia is Ginger (*Zingiber officinale*). Ginger has three major clones, they are white ginger, small white ginger, and red ginger [9].

The methanol extract of red ginger has antimicrobial activity against *Streptococcus pyogenes*, *S. aureus*, *E. coli* and *P. aeruginosa* which is higher than the methanol extract of white ginger [10]. Research by Hertiani *et al*, (2011) showed that the essential oil of red ginger has antibiofilm activity against *Streptococcus mutants* that causes dental plaque [11]. Red ginger essential oil has 54 compounds. Most of the essential oil contains monoterpenes (81.9%) which mainly consists of camphene (14.5%), geranil acetate (13.7%), geranial (14.3%), neral (7.7%), geraniol (7.3%), and 1,8 cineole (5.0%) and has antibacterial activity against gram-positive bacteria (*B. licheniformis*, *B. spizizenii*, and *S. aureus*) and gram-negative bacteria (*E. coli*, *K . pneumoniae*, and *P. stutzeri*) [12].

The red ginger essential oils were used in this research. The aim of this study was to examine the antibacterial and antibiofilm activity of the red ginger against *S. epidermidis*. Antibacterial activity was determined using agar diffusion method while biofilm inhibition and degradation activity were determined using the microtiter plate assay. Crystal violet were used to stain the biofilm and optical density (OD) was measured at λ 595 nm.

2. Materials and Methods

a. Plant materials

Dried rhizome of *Zingiber officinale var rubrum* were collected on April, 2014 from Yogyakarta, Indonesia.

b. Isolation of the essential oils

Approximately 5 kg of dried rhizome was hydro-steam distilled for 5 hours. The appearances of essential oils yielded were observed. Those oils were sealed and kept in dark glass vials for further analyses. The essential oil was added anhydrous sodium sulfate until saturated then calculated the yield percent.

$$\text{Yield (v/b)} = \frac{\text{volume of essential oil}}{\text{weight of initial crude}} \text{x100 \%}$$

c. Preparation of Sample

The essential oils were dissolved in 10% aqueous dimethylsulfoxide (DMSO) with Tween 80 0.5% [12]. Gentamicin 0.0276 mg/ml was used as positive control.

d. Bacteria and Culture Condition

S. epidermidis was used from Microbiology and Biotechnology Laboratory, Pharmacy Faculty, Jember University. It was resistant to 54 antibiotic. The culture of bacteria were grown in Blood Agar for 24 h at 36.6°C. A McFarland standard 0,5 was used to adjust inoculum density in NaCl solution for antibacterial assays. Bacteria for biofilm assays were grown in Tryphton Soy Broth (TSB) and inoculums density was adjusted to McFarland standard 5.

e. Antibacterial Activity Assay

Antibacterial assay was done by the well diffusion method. It was performed using an 24 h culture at 37°C in 10 ml of Mueller Hinton Broth. Five hundred microliters of the bacterial suspensions were poured the plates containing Mueller-Hinton agar. Five wells was made on the Mueller Hinton Agar. Each well was impregnated with the essential oil suspension and gentamicin 0,0276 mg/ml as positive control [14].

f. Biofilm Inhibition Assay

Biofilm Inhibition Assay was adapted from [15]. Essential oil suspension, bacterial suspensions, and TSB were put on flexible U-bottom 96-well polystyrene plates and were incubated for 18-24 h growth at 36.6°C. Gentamicin 0.0276 mg/ml was used as positive control. After the end of incubation, the contents of the wells were aspired, rinsed 3 times with distilled water, and were fixed for 10 min. Then, 125 µL of 0,4% crystal violet stain was added to the wells and incubated for 15 min. The excess stain was rinsed off with tap water. A 200 µL ethanol 96% was added to each wells, incubated for 15 min and then 150µL was transferred to a flat bottom 96-well plates. Optical density readings were taken using micro plate reader at 595 nm.

g. Biofilm Degradation Test

The bacterial suspensions were put on on flexible U-bottom 96-well polystyrene plates and were incubated for 18-24 h growth at 36.6°C. After 24 h incubation at 36.6° C, the contents of the wells were aspired. Essential oil suspension and TSB were added each well and were incubated for 18-24 h growth at 36.6°C. Gentamicin 0.0276 mg/ml was used as positive control. After the end of incubation, the next procedure was done as the biofilm inhibition method.

The formula used to calculate the inhibition and degradation were as follows [15]:

$$\%\text{inhibition} = 1 - \frac{(\text{OD sample} - \text{OD Blanko Sample})}{(\text{OD Vehicle} - \text{OD Blanko Vehicle})} \times 100\%$$

Annotation:

OD sample	:	OD essential oil + bacterial suspension
OD Blanko Sample	:	OD essential oil + physiological NaCl
OD *Vehicle*	:	OD solvent control+ bacterial suspension
OD blanko *Vehicle*	:	OD solvent control+ physiological NaCl

The result of antibacterial assay was analyzed using one way ANOVA test and were continued with LSD test while antibiofilm assay was analyzed using Kruskal Wallis test and continued with Mann-Whitney test. IC_{50} was calculated for Inhibition biofilm and EC_{50} was for degradation biofilm. Both of them were analyzed using Probit.

3. Result and Discussion

h. Result

Distilled essential oils were characterized with various parameters shown in Tab. 3.1. The result of antibacterial assay was shown in Tab. 3.2 while The result of inhibition and degradation biofilm assay were shown in Tab. 3.2 and Tab. 3.3, respectively.

Table 3.1 Characteristic of Distilled Essential Oil

Parameter	Red Ginger Essential Oil
Color	Yellow orange
Smell	Typical ginger
Taste	Spicy
Mass	0,9093g/ml at 27^{o}C
Bias Index	1,483 at 27^{o}C

Table3.2 The result of Antibacterial Assay

Concentration of essential oil suspension	Inhibition Zone Mean (mm) ± SD (n = 5)
50%	8,650 ± 0,285[a]
60%	9,500 ± 0,306[b]
70%	10,15 ±0,335[c]
80%	11,05 ±0,41[d]
Positive control	13,95 ±0,737[e]

Annotation: different notation (a, b, c, d, e, f) indicate significant differences between treatments with LSD test ($p< 0,05$).

Table 3.3 The result of Biofilm Inhibition Assay

Concentration of essential oil suspension(v/v)	Inhibition Biofilm Mean (%) ± SD (n=3)
50%	39 ± 3,0[a]
60%	58 ± 5,3[b]
70%	79 ± 5,3[c]
80%	93 ± 3,0[d]
Positive control	60 ±3,0[b]

Annotation: different notation (a, b, c, d, e) indicate significant differences between treatments with Mann Whitney test ($p< 0,05$).

Table3.3 The result of Biofilm Degradation Assay

Concentration of essential oil suspension (v/v)	Degradation biofilm Mean (%) ± SD (n=3)
50%	37 ± 2,5[a]
60%	51 ± 4,4[b]
70%	62 ± 2,5[c]
80%	76 ± 2,5[d]
Posotove control	43 ± 3,4[e]

Annotation: different notation (a, b, c, d, e, f) indicate significant differences between treatments with Mann Whitney test ($p<0,05$).

4. Discussion

Based on test results, red ginger essential oil inhibited growth of *S. epidermidis* bacteria. It was noticed from the formation of a clear zone around the wells on Mueller Hinton medium.

Antibacterial activity of red ginger essential oils might be caused by essential oil lipophilicity . Therefore, it would pass through the cell wall, cytoplasmic membrane, and damage the structure of the bacterial cell and make it more permeable. Bacterial membrane permeability closely related to missing ions, decreasing of membrane potential, proton pump damage, and absence of ATP. Then, cytoplasm coagulation occurs followed by destruction of lipids and proteins by essential oils. The destruction of the cell walls and membranes may lead to macromolecules leakage that would cause lysis [16].

In this research, red ginger essential oil was also known to have the activity to inhibit and degrade biofilms. IC_{50} in the biofilm forming inhibition was 55.270% (v/v) and EC_{50} in biofilm degradation was 58.986% (v/v).

Based on the research, Red ginger shows a better antibacterial activity than *lempuyang wangi*. *Lempuyang Wangi* showed the antibacterial and antibiofilm against *S. epidermidis* [17]. However, red ginger has a lower antibiofilm activity than *lempuyang Wangi*. IC_{50} and EC_{50} of Lempuyang wangi were 40.9205% and 48.064%, respectively. Due to different activity, both red ginger and *Lempuyang Wangi* would be a good combination as antibacterial and antibiofilm for the next research.

5. Summary

Red ginger essential oils have antibacterial activity and S. epidermidis antibiofilm. The different levels of essential oils suspense i.e. 50%, 60%, 70%, 80% showed the different antibacterial activity and antibiofilm against S. epidermidis.

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Antibacterial and Antibiofilm Activities of Zingiber aromaticum Essential Oil against Staphylococcus epidermidis

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Abstract - *Staphylococcus epidermidis* may cause nosocomial infections. One of problems in the treatment of infections caused by *S. epidermidis* is biofilm formation. Medicinal plants have been used as traditional treatments for numerous human infections. This research proposed to find out antibacterial and antibiofilm effect of essential oil from *Zingiber aromaticum* against *S. epidermidis* at various concentrations 50, 60, 70, and 80 % (v/v). Antibacterial activity was determined by agar diffusion methods. Antibiofilm activity was measured by microtiter plate biofilm. Biofilms were stained by 0.4 % crystal violet and quantified by measuring the absorbance at 595 nm. This study showed that *Z. aromaticum* essential oil has antibacterial and antibiofilm activity on *S. epidermidis*. The concentration required to inhibit 50% biofilm formation (IC_{50}) and to degrade 50% of biofilm (EC_{50}) were 40.92 % (v/v) and 48.06% (v/v) respectively.

Keywords: nosocomial infections**,** *Zingiber aromaticum* essential oil, antibacterial, antibiofilm, *S. epidermidis.*

1. Introduction

Nosocomial infection is the infection acquired from hospitals or when the patient was hospitalized [1]. This infection occurs in at least 3 x 24 hours since the treatment started, and not an infection as the continuation of treatment before [2].

Nosocomial infection is quite worried because it keeps increasing. Based on data collected retrospectively for 2 years in RSAB Harapan Kita, Jakarta, there were an escalation in the incidence of infection of 9 % on day 1 to 63 % at 3-5 day care in the NICU and there were 56 mortality due to sepsis nosocomial by 6600 total births in that period [3]. One of the causes of nosocomial infections is the *Staphylococcus epidermidis* [4].

There were five bacteria found on patients catheter hospital at RSUP Dr. M. Djamil Padang. They were *S. aureus, S. epidermidis, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumonia* [5]. Approximately 60 % of *S. epidermidis* strains are resistant to nafcillin [6]. *S. epidermidis* is also resistant to meticilin, aminoglycosides and macrolides, as well as most other antibiotics such as tetracycline, chloramphenicol, and clindamycin [7].

A problem in the treatment of infections caused by *S. epidermidis* is the presence of biofilm [8]. Biofilms are complex communities of interacting bacteria and secrete a protective products such as polysaccharides, proteins, nucleic acids, and other compounds substances [9]. Various bacteria makes biofilms as protection [10]. High rates of infection indicates the need for new antimicrobial agent especially from natural materials because of its availability and a wide variety of active substances. *Zingiberaceae* is one of the plants family that contains essential oils. Most of the essential oils contains terpene compounds and derivatives. Components monoterpene compounds such as geraniol, terpinen-4-ol, menthol, linalol, menthon, camphor, 1,8-cineol, D-limonene and α-pinene has antibacterial activity against *Haemophilus influnzae, Streptococcus pyogens, S. pneumoniae, S. aureus* and *Escherichia coli* [11].

One of the plants from *Zingiberaceae* is *Zingiber cassumunar* that has inhibitory effect on some bacteria such as *B. cereus, S. aureus, E. coli,* and *P. aeruginosa* [12]. *Zingiberaceae* also has activities antibiofilm activity such as *Zingiber officinale* [13] and *Boesenbergia rotunda* [14].

Zingiber aromaticum belongs to *Zingiberaceae*. It contains α-pinene (7,86%), camphene (31,27%), ß-pinene (2,39%), β-cis-osimen (3,41%), α-terpinene (0,84%), 3-kalarene (1,80%), 1,8-sineol (6,36%), 4-kalarene (1,25%), β-linalool (14,16%) [15]. The tests that have been done to *Z. aromaticum* showed the antibacterial activity on *Streptococcus beta hemolyticus* [16]. Other studies also showed that *Z. aromaticum* essential oil inhibited the activity of *B. cereus bacteria, S. typhi,* and *P. aeruginosa* bacteria[17].

The purpose of this research was to determine the potential of *Z. aromaticum* essential oils as antibacterial and antibiofilm against *S. epidermidis*

using the diffusion method for antibacterial test and microtiters plate biofilm assay for antibiofilm test.

2. Materials and Methods

j. Materials

DMSO (dimethyl sulfoxide), sterile distilled water, physiological NaCl, 0.4% crystal violet, tween 80, ethanol 96%, Mc Farland 0.5; Mc Farland 5. Test bacteria used was *S. epidermidis* that has been resistence to 53 antibiotics. Bacterial medium used were *Mueller Hinton* (Merck), *Blood agar*, *Trypthon Soy Broth* (TSB) (Merck), while the enricher medium is *Nutrient broth* (NB). Positive control was gentamicin injection (Merck). *Z. aromaticum* simplicia was collected in Ngantang, Malang.

k. Instruments

Microplate reader (Dialab elx 800), micropipet (Socorex^{R}), autoclave, hydro-steam distillation, refractometer Abbe (Atago, Jepang), caliper, microplate flat-bottom and flexible U-bottom PVC 96 wells, incubator (Clifton), Laminar Air Flow cabinet (Airtech).

l. Essential Oil

About 2.5 kg of *Z. aromaticum* simplicia was hydro-steam distilled for 5 hours. The yield of essential oils was separated from the water. Essential oils was calculated its yield (%).

$$\text{Yield (\%)} = \frac{volume\ of\ essential\ oil}{weight\ of\ initial\ crude} \text{x}100\%$$

The essential oil was sealed and kept in dark glass vials for further analyses. Essential oil was tested for its organoleptic, refractive index, and density. Refractive index and density is the physical properties of essential oils which will ensure essential oils authenticity [18].

m. Bacteria and Culture condition

S. epidermidis was collected in Microbiology laboratory, pharmacy faculty that has been resistence to 53 antibiotics. It was grown in nutrient broth for 24 hours at 37 ^{0}C. The cultures were adjusted to approximately Mc farland 0.5 with NaCl solution for antibacterial assay. Mc farland standard 5 was used to inhibition and degradation biofilm assay.

n. Antibacterial Assay

Antibacterial activity was done by the well diffusion. The culture was inoculated to Mueller Hinton agar at 37°C for 24 hour. One thousand microliters of the cultures suspensions were spread over the plates containing Mueller-Hinton agar. The essential oils were dissolved in 10% aqueous (DMSO) with Tween 80 (0.5% v/v for easy diffusion) [19]. Essential oil concentration made were 50, 60, 70, and 80 % v/v. Gentamicin (0,00034 mg/ml) was used as reference control. All petridishes were sealed with sterile laboratory parafilm to avoid eventual evaporation of the test samples. The plates were left for 30 min at room temperature to allow the diffusion of oil, and then they were incubated at 37°C for 18 hour. After the incubation period, the zone of inhibition was measured with a calliper. Studies were performed in five replication, and mean value was calculated. The means were analysed by Kruskal-Wallis followed by Mann Whitney test. The results were expressed as mean ± SD. P values < 0.05 were considered as significant.

o. Biofilm Inhibition Assay

Biofilm inhibition assay was modified from Ardani *et al* (2010) [20]. Serial dilutions of sample solutions were put on flexible U-bottom 96-well polystyrene plates and were incubated for 18-24 hour growth at 37°C. Blank samples were obtained by performing the same treatment as for samples, only without cells added. All tests were performed at least in triplicate. After 18-24 hour incubation, the contents of the wells were aspired, rinsed 3 times with distilled water, and were fixed for 10 min. Then, 125 µl of 1% crystal violet stain was added to the wells and left for 15 min. The excess stain was rinsed off with tap water. A 200 µl methanol was added to each wells, left for 15 min and then 150µl was transferred to a flat bottom 96-well plates. Optical density readings were taken using micro plate reader at 595 nm. To account for the effect of the essential oils color, a formula for calculating percent inhibition was used

IC_{50} was determined as a concentration of sample which inhibited 50% of biofilm formation in comparison to vehicle controls. It was calculated by using probit analysis.

p. Biofilm Degradation Assay

Biofilm inhibition assay was modified from Ardani *et al* (2010) [20]. EC_{50} Serial dilutions of sample solutions were put on flexible U-bottom 96-well polystyrene plates. Blank samples were obtained by performing the same treatment as for samples, without cells added. All tests were performed at least in triplicate. After 24 hour incubation at 37 °C, the contents of the wells were aspired. Serial dilution of essential oils in media were added to the wells and incubated for another 24 hour in 37 °C incubator. Further treatment was as done for biofilm inhibition assay. Effect of essential oils on the growth of *S. epidermidis* evaluated using microplate reader at optical density 595 nm. EC_{50} was determined as a concentration of sample which degrade 50% of mature-biofilm formed in comparison to vehicle controls. Mature biofilm formed for 24 hour. Data was calculated by using probit analysis.

The formula used to calculate the inhibition and degradation are as follows:

$$\%\text{inhibition}=1-\frac{(OD\text{sample}-OD\text{Blanko Sample})}{(OD\text{Vehicle}-OD\text{Blanko Vehicle})} x\ 100\%$$

Annotation
OD sample : OD of essential oil + bacterial suspension
Sample : OD of essential oil + physiological NaCl
OD Vehicle : OD of solvent control+ bacterial suspension
Vehicle : OD of solvent control+ physiological NaCl.

3. Results and Discussion

q. Results

Distilled essential oils were characterized with various parameters shown in Tab. 3.1. Activity of essential oils were seen by performing two tests, antibacterial (Tab. 3.2) and antibiofilm. Antibiofilm test consisted of inhibition test and degradation test (Tab. 3.3). The yield of essential oil was 0,885% (ml/g).

Table 3.1 Characteristic of Distilled Essential Oil

Parameter	*Z. aromaticum* Essential Oil
Color	Less clear
Smell	Typical aromatic of *Z. aromaticum*
Taste	Bitter
refractive index	1,495
Density	0,9054 (g/ml)

Table 3.2 Antibacterial assay result

The concentration of essential oil emulsion (v/v)	Inhibition zone (mm) Mean ± SD
50%	3.332 ± 0.237^{a}
60%	4.200 ± 0.504^{b}
70%	5.600 ± 0.595^{c}
80%	7.266 ± 0.493^{d}
gentamicin	$6,134 \pm 0,379^{c}$

Note: number was followed by different letters within a column indicate significant differences based on difference test at α level = 0.05, n= 5

Table 3.3 Results of biofilm inhibition assay and degradation assay.

Concentration % (v/v)	biofilm inhibition (%)	biofilm degradation (%)
50	$71,111 \pm 3,849^{a}$	$63,888 \pm 4,811^{a}$
60	$82,222 \pm 3,849^{b}$	$69,444 \pm 4,811^{a,c}$
70	$84,444 \pm 3,849^{b}$	$72,222 \pm 4,811^{a,c}$
80	$86,666 \pm 6,666^{b}$	$83,333^{b}$
gentamicin	$86,666^{b}$	$77,777 \pm 4,811^{c}$

Note: inhibition and degradation biofilm assay were determined mean ± SD. Number was followed by different letters within a column indicate significant differences based on difference test at α level = 0.05, n= 3

r. Discussion

The test results showed that the essential oil of *Z. aromaticum* was able to inhibits the growth of *S. epidermidis*. The order of essential oils concentration having antibacterial activity were 80 % (v/v) > 70 % (v/v) > 60 % (v/v) > 50% (v/v). The antibacterial properties of essential oils and their components have been reviewed in the past and the mechanism of action has not been studied in great detail [21]. Essential oils is suggested to inhibit the growth or kill bacteria through the process disruption on cell wall formation, destruction of the cell membrane, and inhibition of the enzyme or annihilation of genetic material in bacteria. This mechanism does not happen separately, but there are several mechanisms that occur as a result of other mechanisms [22].

Antibacterial activity of *Z. aromaticum* essential oils against *S. epidermidis* was not too great compared to the essential oil of orange leaves (*X Citrofortunella microcarpa (Bunge) Wijnands*). Essential oil of orange leaf has 23 mm inhibition diameter at concentration of 3.25% [23], while the inhibition diameter of *Z. aromaticum* at 50% concentration was 3.332 ± 0.237 mm. The great difference is prrobably due to *S. epidermidis* in this study was resistant to 53 antibiotics.

The ability of biofilm inhibition and degradation of compounds closely related with compound's ability to penetrate the layer of *extracellluler polimeric substance* (EPS) or the ability of the compound to degrade biofim [24]. Essential oils helps to destroy biofilm adhesion [25]. Inhibition and degradation assay of Z. aromaticum essential oil was higher than that of red ginger. IC_{50} inhibition test was 40.920% (v/v) while the degradation test EC_{50} is 48.064%(v/v). This result was bigger than result test from red ginger. IC_{50} in the biofilm forming inhibition was 55.270% (v/v) and EC_{50} in biofilm degradation was 58.986% (v/v) [26]. Thus, research of *Z. arommaticum essential oil* need further study to knowing mechanism of each compounds as antibiofilm.

4. Summary

Z. aromaticum essential oils have antibacterial and antibiofilm activity against *S. epidermidis*. The higher concentration have higher antibacterial activity. Inhibition activity have greater than degradation activity.

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Encapsulation of Antioxidant Extract Coffee Cherries by Using Arabic Gum Combined Tapioca Oxidation as Capsuled Material

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Abtract - The obyective of this research was to study the ratio of tapioca oxidation and Arabic gum as capsule materials to characterize antioxidant encapsulation, the content and activity of antioxidant. The extract of antioxidant coffe cherry produced with etanol-aquades (1:1) as a solvent. This extract, tapioca oxidation and Arabic gum soluted on aquadest with 20 and 25% suspended. As capsule material use Arabic gum and tapioca oxidation with ratio (90:10; 80:20; 70:30) and 100% Arabic gum as a control. The result showed that extract antioxidant of coffe cherries encapsulated with 25% suspention (1:4) has the best of content and antioxidant activity .Ratio of tapioca oxidation: Arabic gum (10:90) has pholifenol, anthocyanine, Vitamine C, antioxidant maximal, water content and higroscopisity minimal.

Key word : encapsulation, antioxidant extract, Arabic gum, tapioca oxidation, stability oxidation

1. Introduction

Encapsulation is a process entrapped sensitife component by polimer protection as agent of encapsulation. Then the component protected of reaction wich destruct and suffer environtment [1]. Spray drying method is a process of encapsulation for produced powder with high antioxidant activity. Generally used Arabic gum as carrier agent [2,3,4] because high dissolved in water with low viscosity.

Oxidation starch is degradation the polymer which caused this starch dissolved on water, in aline with carbonyl and carboxyl content increase [5].This is suitable used as capsule material.

In other to capsule with good charateritics, the treatment by some variation of component capsule material (ratio of starch oxidation : Arabic gum) and consentration of antioxidant extract. Expentantly encapsulation procces is not destruction antioxidant activity coffee cherries.

2. Methodology

Raw material of extract antioxidant is the fruit of before ripe Arabic coffee cherries from Durjo estate, Jember, East Java Indonesia. Arabic gum from Bratachem Surabaya, tapioca oxidation maked with hydrogen peroxide.

Before ripe coffee cherries extract, but after washing the fruit, separated the pulp and seed.15 g the pulp blend with 75 ml solvent (contained of aquadest: ethanol 97% 1:1 and 15 % citric acid V/V), maceration during 15 minute and filtered the solution. The residue replicated extracted 2 times until colorless. Then centrifugated on 4000 rpm during 20 minute. After that the filtrate to concentrated by rotary evaporator on 400C until the volume extract 75 ml. Mixture this extract, tapioca oxidation and Arabic gum dissolved on aquadest with 20% and 25% suspension (ratio extract : material of capsule 4:1 and 5:1).Some variation of capsule material (ratio tapioca oxidation : Arabic gum 10:90; 20:80 and 30:70) and then spray drying use spray dryer Lab Plant SD-05 (Hudderfiled,English) Lab scale with 1,5 mm diameter of the pipe and space of spray 500 mm x 215 mm. feed flow 15 g/minute, temperature out and in this spray 78 ^{0}C and 110^{0}C.

The data was analyzed by descriptife test and showed the table of chemical and physical characteristics, namely:

Chemical characteristic: total anthocyanin (differential method [6]), Polyphenol total (Follin ciocalteau [7]), carotenoid total [8], Ascorbic acid (titration method [9]), Antioxidant activity (DPPH method [10]).

Physical characteristic: rendemen, water content (oven method [9]) , capsule color (color reader), size of capsul (difractometre laser light mastersizer X, Malvern, UK) and higroscopisity [11].

3. Result and Discussion

Anthocyanine, polyphenol, carotenoid, ascprbic acid content and antioxidant activivity showed on table 1. Encapsulation use capsule material : antioxidant extract (1:4) resulted antioxidant content (anthocyanine, poliphenol, carotenoid, ascorbic acid) and antioxidant activity higher then ratio 1:5. This showed capsule material more good entrapped antioksidan on emulsion system. Concentration tapioca oxidation more high followed anthocyanine,

polyphenol, carotenoid and ascorbic acid increase. Because tapioca oxidation can entrappe component bioactive more good.

Encapsulation extract of antioxidant : material of capsule (1:4) has rendement, water content, size and higroscopisity more high then capsule with ratio extract : capsle material 1: 5. Tapioca oxidation 10% (10:90) have rendemen, water content, size and higrocopysity high.

Table 1.Chemical characteristic of antioxidant capsule

Treatment	Anthocyanine (mg/100 g)	Polyphenol (mg/100 g)	carotenoid (mg/100 g)	Ascorbic acid (mg/100 g)	Antioxidant activity (%)
Extract antioxidant	2.825 ±0.26	24109 ± 4.61	34921.8 ± 2.52	2288.0 ± 0.24	60.25 ± 0.02
S5T0	1.774 ± 0.06	14375 ± 1.094	5592.25 ± 0.19	7770.4 ± 3.26	82.97 ± 0.48
S5T10	1.044 ± 0.30	4250 ± 1.09	7376.87 ± 0.82	8228 ± 0.44	87.10 ± 0.17
S5T20	0.730 ± 0.10	7468.75 ± 0.78	8144.77 ± 0.31	8166.40 ± 0.70	85.60 ± 0.36
S5T30	0.418 ± 0.01	10671.87 ± 1.56	9733.38 ± 4.5	8113.60± 0.70	83.98 ± 0.19
S4T0	1.669 ± 0.10	10984.375 ± 0.94	16618.46 ± 0.20	7669.20 ± 2.77	81.58 ± 0.15
S4T10	0.835 ± 0.03	23265.63 ± 0.63	6044.94 ± 0.82	8113.6 ± 0.35	87.22 ± 6.70
S4T20	0.626 ± 0.02	19750 ± 3.28	7223.20 ± 1.54	8043.2 ± 0.88	85.74 ± 0.59
S4T30±	0.418 ± 0.04	15468.75 ± 0.82	10143.20 ± 1.01	7972.8 ± 0.88	83.87 ± 0.06

Table 2.Phisical characteristic of antioxidant capsule

treatment	Rendement (%)	water content (%)	colour (H)	size (µm)	higroscopisity (%)
S5T0	5.2 ± 0.10	16.30 ± 0.95	82.66 ± 0.14	6.9 ± 0	29.3 ± 0.02
S5T10	2.9 ± 0.02	14.83 ± 0.33	81.96 ± 0.06	7.1 ± 0	28.4 ± 0.06
S5T20	4.9 ± 0.02	6.70 ± 0.60	81.33 ± 0.37	8.3 ± 0	23.1 ± 0.04
S5T30	5.5 ± 0.01	11.70 ± 0.91	82.03 ± 0.12	7.1 ± 0	22.7 ± 0.02
S4T0	5.8 ± 0.02	12.00 ± 0.40	81.29 ± 0.26	12.1 ± 0	33.5 ± 0.01
S4T10	5.9 ± 0.03	18.50 ± 0.40	81.03 ± 0.19	8.3 ± 0	32 ± 0.01
S4T20	4.5 ± 0.01	9.56 ± 0.66	80.0 ± 0.19	5.2 ± 0	32.1 ± 0.01
S4T30	5.7 ± 0.02	11.30 ± 0.96	81.61 ± 0.18	5.2 ± 0	32.4 ± 0.03

4. Conclusions

Antioxidant of coffee cherries encapsulation with 25% suspenstion (ratio extract : capsule material 4:1) has antioxidant ontent and activity maximal the wee the carotenoid and polyphenol. Ratio tapioca oxidation :Arabic gum (10:90) has plyphenol, anthocyanine, ascorbic acid and antioxidant activity the highest, water content ang higroscopisity high.

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Anti-Quorum Sensing Activity of Some Malaysian Medicinal Plants

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Abstract **-** *Parkia spaciosa, Centella asiatica* and *Piper nigrum* are well-known plants of tropical region with many reported medicinal importance. Anti-quorum sensing activities of the selected plants were tested on *Chromobacterium violaceum* ATCC 12472 using agar well diffusion assay. The results showed observable zones of inhibition for the extract from the two solvents except for the methanolic extract of *P. nigrum*. When the concentrations of the extracts were varied from (200mg/ml - 800mg/ml) the anti-QS activity was observed to be concentration dependent and significance of anti-QS activity was tested with one sample t-test as ($P<0.05$). Minimum of QS inhibition concentration was established for each of the plant extracts. Zones of inhibition were observed to be significantly wider with ethanolic extracts.

Keywords: Parkia spaciosa, Centella asiatica, Piper nigrum Quorum sensing, Anti-quorum sensing.

1. Introduction

Few of the known medicinal plants in Malaysia are *Parkia spaciosa, Centella asiatca* and *Piper nigrum* [1],[2].

Parkia spaciosa (petai), is of Mimosa subfamily whose seeds are consumed as a condiment or vegetable with rice [3]. *P. spaciosa* has chemical and medicinal compounds [4], and used for the treatment of certain bacterial diseases on kidney, ureter , urinary bladder [4] and have anticancer activity [5] and the compounds of sterols in *P. spaciosa* have been used for hypoglycemic effect in rats [6].

Centella asiatica (pegaga) is one of the medicinal plants of tropical regions [7],[8]. *C. asiatica* belongs to the family Apiaceae [8] and became popular with its reputation as a brain stimulant [6]. *C. asiatica* has been used in wound healing, atherosclerosis, mental disorder, fungi and bacteria infections and even cancer [7].

Piper nigrum or black pepper belongs to the family of Piperaceae [8]. Black pepper is a taste enhancer in the food and therapeutic purposes [9]. They also have good antibacterial activities against both Gram negative and Gram positive bacterial [10], and it also possesses antioxidant activity.

Anti-quorum sensing disrupts or interferes the processes employed by bacteria to communicate without killing them. They use this process to invite themselves to a specific place until their population is enough to express this effect [11],[12]. Prevention of these bacteria communication may be the novel means of rendering them inactive as this will not impose any pressure for them to form resistance. Searching for quorum sensing inhibiting compounds has therefore become paramount, especially in natural products with good medicinal history like *P. spaciosa, C. asiatica* and *P. nigrum.*

2. Materials and Methods

2.1 Plant extracts

P. spaciosa C. asiatica and P. nigrum were collected and washed with ordinary tap and sterile water; dried and made to powder. Powder of 50g each were separately added to 99.9% methanol (500ml) in three different bottles; an equivalent of 100g dry weight/L. The mixture was allowed to stand for 5days with intermittent shaking on a shaker (150rpm). After which vacuum filtration was done with, Whatman No 1 filter paper. Filtrate was collected and the remaining solvent was evaporated to dryness. The crude extract was reconstituted in 1% DMSO and stored in the chiller for further use. The same procedure as above was used to obtain the ethanolic extract (70% ethanol).

2.2 Biomonitor organism

C. violaceum ATCC 12472 was maintained in glycerol [13] and stored at -80° C. It was then grown at 30ºC on LB agar for 24h before growing in LB broth for 18h in a shaking incubator at temperature of 30ºC.

2.3 Anti-quorum sensing bioassay

Five milliliters of molten soft top agar (1.3g agar No 3, 3g tryptone water) was seeded with 100µL of freshly grown culture of *C. violaceum*. This was homogenized by gentle mixing and poured immediately over the surface of the solidified LB agar plates as an overlay and allowed to solidify. Aseptically, wells of 5mm in diameter were made on the solidified agar of each plate. Each of the wells were then filled with 50µL of crude extract from the selected plants (*P. spaciosa, C. asiatica* and *P.*

nigrum) that has been reconstituted in 1% DMSO at different concentrations (200mg/ml, 400mg/ml, 600mg/ml, 800mg/ml). The negative control wells were filled with 50µL of LB broth. The plates were incubated at the temperature of 30º C for 24h. Anti-quorum sensing activity was investigated by measuring the inhibition zones (mm) of purple color pigment violecein [14].

2.4 Test for Minimum of QS inhibition concentration

Plant extracts that showed QS inhibition were further evaluated to determine minimum of QS inhibitory concentration using agar well diffusion assay. Plant extracts were serially diluted to lower concentrations. Agar and the bacteria culture were prepared by the same method of [14]. Serially diluted extracts were tested on the biomonitor strain.

2.5 Anti-quorum sensing activity and antibacterial effect

Nature of the ring around the wells was used to establish whether the effect observed was antibacterial or anti-quorum sensing. Disc diffusion assay (using tetracycline disc) was performed; the agar and the bacteria culture was prepared the same way as for the anti-QS assay. Instead of making wells on the prepared agar plate, tetracycline discs were carefully placed on the agar and incubated at 30° C for 24h.

2.6 Statistical analysis

Data obtained for each of the selected plants were analyzed, mean difference and significant level were obtained with one sample t-test using statistical package of social sciences (SPSS). Statistical significance was established at $P < 0.05$.

3. Results

Table 1 displays all the results of the anti-QS bioassay of the methanolic and ethanolic extracts of the selected plants. The data showed that higher zones of inhibition were produced by ethanolic extracts. The zones of inhibition produced by ethanolic and methanolic extracts of *P. spaciosa* and *C. asiatica* were statistically significant ($0.000 < 0.005 < 0.05$ and $0.000 < 0.004 < 0.05$ respectively) when analyzed with one sample t-test; and that produced by ethanolic extract of *P. nigrum* was also significant $0.004 < 0.05$. As shown in figure 1 & 2, the zone of inhibition produced in both extract increases steadily as the concentration increases. Values of minimum of QS inhibition concentrations were presented in Table 2.

In a disc diffusion assay of tetracycline, halo of clear zone was observed under a higher magnification this was compared with those produced around the wells containing the plant extracts under the same magnification. It was observed that only the ethanolic extract of P. spaciosa produced such halo, even though there are indications of turbid halo at certain part of the ring around the well containing the extract. It can only be assumed from the result of this juxtaposition that ethanolic of *P. spaciosa* exhibited antibacterial effect along with anti-QS activity.

Table 1. Zones of inhibition produced by the ethanolic and methanolic extrtacts

Plant extracts		**Zone of inhibition (mm) at different concentration**			
Plants	**solvents**	**200mg/ml**	**400mg/ml**	**600mg/ml**	**800mg/ml**
P. spaciosa	methanol	8.67 ± 0.2	11.33 ± 0.8	13.33 ± 0.2	16.50 ± 0.2
	ethanol	19.17 ± 0.2	20.33 ± 0.3	21.50 ± 0.3	22.83 ± 0.2
C. asiatica	methanol	8.67 ± 0.3	11.67 ± 0.3	14.33 ± 0.3	15.83 ± 0.3
	ethanol	15.17 ±0.3	16.67 ± 0.3	18.37 ± 0.1	19.17 ± 0.3
P. nigrum	methanol	----------	----------	----------	----------
	ethanol	7.17 ± 0.3	7.83 ± 0.3	9.23 ± 0.3	12.17 ± 0.3

Table 2: Minimum of QS inhibitory concentration (mg/ml)

Plant extracts	**Minimum of QS inhibitory concentration(mg/ml)**	**Zone of inhibition (mm)**
***P. spaciosa* (methanolic)**	200	7.10±0.1
***P. spaciosa* (ethanolic)**	100	8.20±0.3
***C. asiatica* (methanolic)**	100	9.67±0.3
***C. asiatica* (ethanolic)**	62.5	12.13±0.1
***P. nigrum* (ethanolic)**	200	7.15±0.3

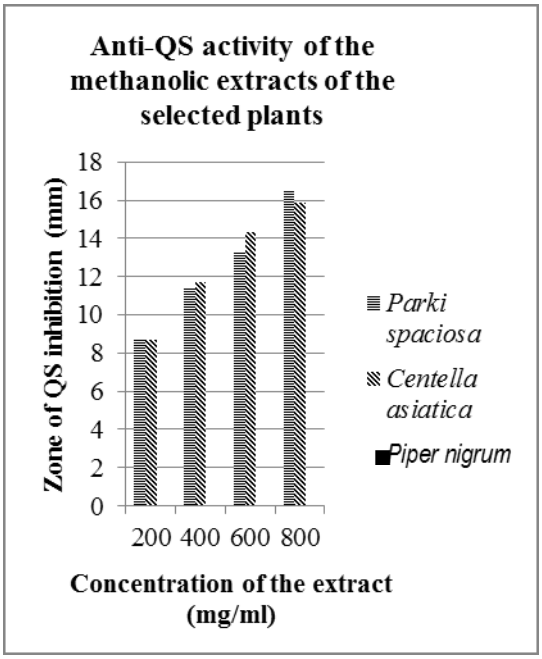

Figure 1: Anti-QS activity of the methanolic extracts of the selected plants

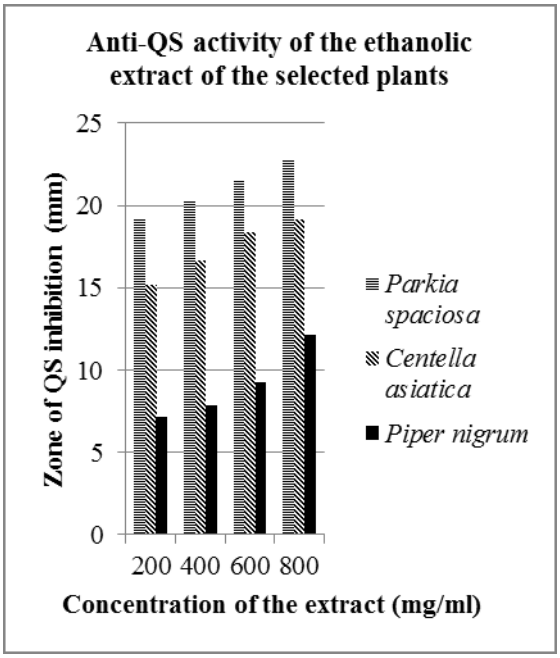

Figure 2: Anti-QS activity of the ethanolic extracts of the selected plants

4. Discussion

The activity observed with ethanolic extracts is more significant than the methanolic extracts (Fig. 1 & 2). Only the methanolic extract of *P. nigrum* did not show violecein inhibition. Ethanolic and methanolic extracts of both *P. spaciosa* and *C. asiatica*, as well as ethanolic extract of *P. nigrum* demonstrated a concentration dependent anti-QS activity.

C. violaceum is one of the bacteria where quorum sensing has been well studied. C. violaceum was used for this screening procedure because of the phenotypic characteristics of purple color pigment of violecein exhibited by this bacterium. QS, as studied in *C. violaceum* is controlled by CviI/CviR circuit (a homologous of LuxI/LuxR) [15]. C. violaceum ATCC 12472 has been used as a biomonitor organism to establish the anti-QS activity of some medicinal plants; few of such plants include *Moringa oleifera* and *Acacia nilotica* [16]; *Laurus nobilis* [17]; and *Chamaesyee hypericifolia* aerial [18].

However, the anti-QS activities of these three plants on *C. violaceum* cannot be considered as paradigm for all other pathogenic bacteria. QS is controlled by different circuit in different bacteria. The autoinducer synthase proteins, autoinducer itself and the protein receptors are different in each bacterium. In fact two signaling pathways have been identified in *Pseodomonas aerugenosa* [19]; therefore identification of inhibitors of QS in this case must be carefully differentiated because the inhibitor of the first pathway may not inhibit the other signaling pathway.

Phytochemical analysis of some of the herbs where quorum sensing inhibition has been established has suggested that generally, anti-QS agents like vannilin, furanones and elligitannins belong to phenolic compounds [20]. Certain bioactive compounds get extracted better with ethanol than methanol; these include flavonols and alkaloids. In the same manner anthocyanin, saponins and flavones are better extracted with methanol [21]. Anti-QS observed with the extracts of these plants may be a direct action of a particular bioactive compound. It may be indirect actions of many compounds or synergistic effect of two or more bioactive compounds.

5. Conclusion

This study has especially established the anti-quorum sensing activity of the three selected medicinal plants i.e *Parkia spaciosa, Centella asiatica* and *Piper nigrum*. Even though other analysis (identification of bioactive compound(s) and mechanism of action) are required for better understanding of this newly attributed features. Future study should include fractionation of the extracts to isolate and characterize the actual compound responsible for the anti-QS activity, so as to understand the mechanism of action.

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Quality and Sensory Properties of Black Tilapia Surimi Gels with Cocoyam Starch Addition

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Abstract - Surimi made from freshwater fish has received low consumer acceptance due to lack of gelling properties. In this study, the potential of cocoyam starch was investigated to improve the gelling ability of freshwater fish surimi made from black tilapia. Two treatments were given to cocoyam: gelatinization and non-gelatinization process. The percentages of cocoyam used were 3%, 6%, 9%, 12% and 15%. The moisture content of surimi added with gelatinized and non-gelatinized cocoyam starches showed values in range of 72.20% – 84.00% and 76.84% – 84.65%, respectively. Black tilapia surimi with 3% addition of gelatinized and non-gelatinized cocoyam starches exhibited the highest moisture content among the samples. Black tilapia surimi with 15% gelatinized and non-gelatinized cocoyam addition were found to have the highest value of water holding capacity (WHC) and hardness property. There was no significant difference observed in springiness values among all the samples. In sensory analysis using Qualitative Descriptive Analysis (QDA), Black Tilapia surimi with 3% of non-gelatinized cocoyam was rated higher as compared to surimi with 3% of gelatinized cocoyam. As a conclusion, the addition of cocoyam starch could improve the physical and sensory properties of Black Tilapia surimi with the percentage of addition less than 3%. It is suggested to study the effect of different level of temperature and time for pre-treatment and incubation process.

1. Introduction

Surimi is a concentrated myofibrial protein extracted from fish flesh obtain by washing the minced meat and mixed with cryoprotectants [1]. Surimi is a primary ingredient to produce imitation products such as fish ball, fish cake and crab stick. The most important properties contributing to consumer preference for surimi products are whiteness and texture [2].

Fresh water fish is extensively studied as an alternative for surimi production as they have low market price, acceptable mince color and textural properties [3]. Although this species shows moderate gel-forming ability, they can be utilized by adapting the parameters of the gelation process [4].

In this study, cocoyam starch will be incorporated in fresh water fish surimi production. Previous studies have shown that starches such as potato and yam improved the gel properties of fresh water fish surimi [5, 6]. The objective of this project was therefore to determine the functional properties and sensory attributes of Black Tilapia surimi with various addition of cocoyam starch.

2. Materials and Methods

2.1 Materials

Black tilapia and cocoyam were bought from night market (Nilai, Negeri Sembilan). All the chemicals and solvents used were of analytical or chromatography grades.

2.2 Methods

2.2.1 Cocoyam starch preparation

Cocoyam was washed, peeled and cut into small cubes prior to drying process. The cut cocoyam was divided into 2 treatments; gelatinization (cooked for 10 minutes at 80°C) and non-gelatinization (no cooking) processes. Both treated cocoyam starch were dried at 95°C for 48 hours in dehydrator (Model 4926T, USA) and ground into fine powder. To obtain uniform size of flour, the cocoyam powder was sieved and packaged in airtight container.

2.2.2 Fish dan Surimi Preparation

Fresh black tilapia was headed, gutted, washed and deboned to obtain fish flesh. The fish flesh was processed into surimi by washing with water at ratio 1:4 (w/v), respectively for three times followed by dewatering step to get the final moisture content of approximately 80%. Sucrose (8%) and NaCl (2.4%) were then mixed to the washed fish flesh. The final step was to incorporate the cocoyam starch at 3%, 6%, 9%, 12% and 15%. The paste was incubated in water bath at two sequence stages: 30 minutes at 60°C and

20 minutes at 90°C. The gel was then allowed to cool immediately for 30 minutes and stored at 4°C prior to analysis.

2.2.3 Functional Properties of Surimi

2.2.3.1 Textural Properties

Textural Profile Analysis (TPA) was carried out using Texture Analyzer (Surrey, UK). The surimi gel was uniformly cut into 24 mm x 24 mm (height x width).

2.2.3.2 Moisture Analyis

The determination of moisture content was done using Official Method of AOAC 925.04 [7].

2.2.3.3 Water Holding Capacity

The analysis of water holding capacity was done according to [8]. A gel sample with a thickness of 0.5 cm was placed between two pieces of filter paper on the top and 3 layers of paper at the bottom. Standard weight (3kg) was placed on the top of the samples for two minutes before remove it. The weight before and after this step was taken and the water holding capacity was calculated according to the following formula;

$$\text{WHC \%} = 100 - \left[\frac{\text{Weight of water loss (g)}}{\text{Moisture content (g)}} \times 100\% \right]$$

2.2.4 Sensory Properties

Quantitative Descriptive Analysis (QDA) was performed on the selected surimi formulations. The analysis was done using ten trained panelists. The attributes tested were hardness, springiness, strength and color.

3. Results and Discussion

3.1 Functional Properties of Surimi

3.1.1 Texture Profile Analysis (TPA)

The result showed that hardness and springiness of black tilapia surimi added with gelatinized and non - gelatinized cocoyam starches increased significantly with the percentage of cocoyam starch (Table 1). These results are in agreement with [3]. Gelation of starch upon cooling leads to this result.

3.1.2 Moisture Analysis

Moisture content of black tilapia surimi treated with gelatinized and non- gelatinized cocoyam starches was in range between 72.20% and 84.65% (Table 2). Generally, the addition of gelatinized cocoyam starch decreased in moisture content as compared to non-gelatinized cocoyam starch. It might be due to starch granule ruptured upon the processing (pre- treatment and incubation).

3.1.3 Water Holding Capacity

Black Tilapia surimi with cocoyam starch addition was found to have higher water holding capacity in comparison to surimi without cocoyam starch (Table 2). The presence of starch in black tilapia surimi contributed to the high values of WHC. Sarker et al. [9] reported that starch act as water absorbant agent in surimi.

3.2 Sensory Properties

Table 3 shows the scores for sensory properties of black tilapia surimi with different level of cocoyam starch incorporation. The test was done to black tilapia surimi with 3% cocoyam starch addition as those samples had the quality looking by the consumers. Surimi with 3% of non-gelatinized cocoyam starch had rated higher as compared to surimi with 3% gelatinized cocoyam starch in terms of hardness, springiness, strength and color.

Table 1: Results of Texture Properties, Moisture Content and Water Holding Capacity (WHC)

Sample	Texture Properties	
	Hardness	Springiness
Gelatinized cocoyam starch		
0%	18.23 ± 1.96^{c}	0.98 ± 0.03^{a}
3%	28.21 ± 0.81^{b}	1.51 ± 1.08^{a}
6%	28.00 ± 1.94^{b}	0.88 ± 0.40^{a}
9%	32.37 ± 2.61^{b}	1.97 ± 1.16^{a}
12%	30.27 ± 2.79^{b}	1.04 ± 0.06^{a}
15%	45.80 ± 1.99^{a}	1.02 ± 0.03^{a}
Non-gelatinized cocoyam starch		
0%	18.23 ± 1.96^{bc}	0.98 ± 0.03^{a}
3%	16.89 ± 5.67^{bc}	1.01 ± 0.04^{a}
6%	18.21 ± 1.09^{bc}	1.00 ± 0.02^{a}
9%	20.62 ± 2.11^{b}	1.00 ± 0.02^{a}
12%	29.44 ± 3.30^{a}	0.99 ± 0.01^{a}
15%	32.53 ± 2.33^{a}	1.00 ± 0.01^{a}

Means with different letter indicate significant different

Table 2: Moisture Content and Water Holding Capacity Values

Sample	Moisture Content	Water Holding Capacity (WHC)
Gelatinized cocoyam starch		
0%	81.00 ± 0.03^{e}	54.84 ± 10.99^{b}
3%	84.65 ± 0.01^{a}	47.19 ± 4.92^{bc}
6%	84.39 ± 0.04^{b}	51.71 ± 9.96^{bc}
9%	82.63 ± 0.02^{d}	48.28 ± 7.18^{bc}
12%	76.84 ± 0.05^{f}	42.91 ± 4.74^{c}
15%	83.35 ± 0.04^{c}	73.49 ± 4.17^{a}
Non-gelatinized cocoyam starch		
0%	81.00 ± 0.03^{d}	56.84 ± 10.99^{bc}

3%	84.00±0.02[a]	47.08±9.55[bc]
6%	83.20±0.05[b]	40.08±12.51[c]
9%	82.60±0.03[c]	54.02±10.53[bc]
12%	80.60±0.06[e]	59.45±9.53[ab]
15%	72.20±0.07[f]	76.33±3.37[a]

Table 3: Sensory Properties Scores

Attributes	3% gelatinized cocoyam starch	3% non-gelatinized cocoyam starch
Hardness	3.00±1.63[b]	4.00±1.33[b]
Springiness	2.50±0.53[c]	4.60±1.51[b]
Strength	1.80±0.79[c]	4.10±129[b]
Color	5.00±1.94[b]	4.50±2.07[b]

Means with different letter indicate significant different

4. Summary

Generally, it can be concluded that the addition of cocoyam improved the functional properties and sensory attributes of surimi made from black tilapia. The ability of starch to absorb water upon gelatinization and incubation processes contributed to this finding. It is recommended to further investigate the effect of different level of gelatinization and incubation time and temperature.

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Reliability Test of Mass Balance Model on Nitrogen Loads at The Rembangan River Jember

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Abstract - The Mass balance model of nitrogen load was based on the continuity and momentum equations. Running the model was inserted the variable input into mathematical models based on differential equations by the finite difference method. Aims this study was to calibration, and validation the model. The coefficient of average monthly runoff (Cb) was defined as the ratio between the peak intensity of rainfall runoff to monthly. In this study, the average runoff coefficient monthly (Cb) was used to calculate the monthly average discharge. Calibration of the runoff coefficient of the monthly average (Cb) was do to find the best value of the runoff coefficient as output the model that was similar to the discharge measurement results in the field. The type of water flow in the river Rembangan Jember could determine with calculated the value of the Reynolds number. From the calculation, the Reynolds number is equal to 1929,821. Thus, the value was smaller than 2000, it was means the type of stream Rembangan including laminar flow. The result of running model was value of the nitrate concentration was 0.6549 mg/liter with calibrate of the reaction coefficient (K) and obtained a value of -1759 x 10-6. The minus sign means that the speed of reaction that occurs in the river Rembangan in July 2010 at a point 2 to decrease or reduction in the value of the reaction rate of 9.88 x 10-5/sec. This shows that the speed of the reactions in the river Rembangan tended to decline and approach the value zero.

Keywords: reliability, mass balance, model, nitrogen

1. Introduction

The nitrogen mass balance model can applicable to predict the load of pollutant which contained in runoff water from fertilization activity in plantation area. Mass balance model was developed based on the continuity equation and the momentum equation [1], the input variables used in the process of running this model are: water density (ρ), velocity of water flow in the direction of the x axis (u), the flow velocity water in the direction of the y axis (v), the concentration of nitrate (c), the x-axis changes tehadap distance (Δx), changes in the distance to the axis y (Δy), the reaction coefficient (K), the speed of the earth's gravity (g), the area of coffee plantations, coffee plant populations, high water level (h), air pressure (Po), the runoff coefficient of the monthly average (Cb) rainfall intensity (I), and the elevation of the river length (L). One example of the input variables at one time running a model that is as is done at the point to two months in July 2010.

In the process of data input that will be used for the process of running the program is the first time the value of the reaction coefficient (K) and the correction coefficient (r) given any numerical value or zero for these parameters will be further calibrated to produce a match between the value of the calculation result model with the value of the results of measurements in the field. Once the process is complete, the data input process is then performed. Aims of this study was to determine the results of the calibration model applied to the Rembangan river Jember.

2. Methods

Inventory and identification of data

This study used primary data. The primary data were taken directly in the field. The water quality testing was conducted in the laboratory of Chemistry Department, Jember University and Laboratory of Environmental Control and Conservation Engineering, Department of Agricultural Technology, Faculty of Agricultural Technology, Jember University. Primary data taken were concentration data of N, velocity and discharge on the measurement of surface runoff.

Data Analysis

The data obtained were made to be data plot to identify trends and patterns that occurred on each type of data. Furthermore, the calibration of the runoff coefficient of the monthly average (Cb) and the calibration of the reaction coefficient (K) by using the trial and error method .

3. Result and Discussion

Calibration Against Runoff Coefficient Monthly Average (Cb)

Before running model must determine type of river water flow. The type of water flow in the Rembangan river Jember could determine with calculated the value of the Reynolds number . From the calculation, the Reynolds number was equal to 1929,821 . Thus, the value was smaller than 2000 , it was means the type of stream Rembangan including laminar flow. The coefficient of average monthly runoff (Cb) is defined as the ratio between the peak runoff to monthly rainfall intensity. In this study, the runoff coefficient of the monthly average (Cb) was used to calculate the monthly average discharge. Calibration of the runoff coefficient of the monthly average (Cb), which aims to find the most runoff coefficient values as output model that was closer to measurement discharge. Control parameter used was the amount of discharge measurements in October 2010 (Figure 1) while the value of the runoff coefficient of the monthly average (Cb) obtained from the calibration runoff coefficient between discharge rate of model calculation results and the measurement discharge in the field in October 2010. (Table 1 and Figure 2).

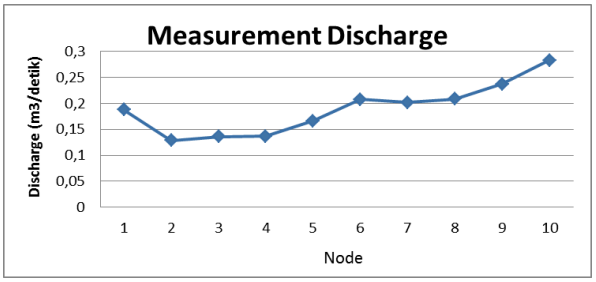

Figure 1. Measurements Discharge on Rembangan River Jember.

Based on the data in Table 1 and Figure 2 shows that the average monthly runoff coefficient (Cb) in October 2010 from point 2 to point 9 tends to fluctuate with the value of the average runoff coefficient of 0.1394. In Figure 2 it can be seen that the overall results of the calibration of the monthly average runoff coefficients (Cb) from point 2 to point 9 shows a similar pattern with the measurements discharge data that was increasing fluctuation in the downstream.

Table 1. Calibration of monthly average runoff coefficients (Cb)

Titik	Q ukur (m^3/dtk)	Intensitas CH rata-rata bulanan **Bulan Oktober** (mm/bulan)	Luas Area A (ha)	Volume rata-rata bulanan = 0.000003858 x I_b x A	Koefisien Limpasan rata-rata bulanan (C_b)
1	0.1881	318	1011	1.240339284	0.151652054
2	0.1285	318	1011	1.240339284	0.103600685
3	0.1364	318	1011	1.240339284	0.10996991
4	0.1371	318	1044	1.280825136	0.107040373
5	0.1662	318	1044	1.280825136	0.129760102
6	0.2084	318	1044	1.280825136	0.162707613
7	0.2021	318	1044	1.280825136	0.157788908
8	0.2084	318	1259	1.544596596	0.13492196
9	0.2371	318	1259	1.544596596	0.153502863
10	0.2834	318	1259	1.544596596	0.183478327
		Rata-rata:		1.347810818	0.13944228

Source: Results of Field Measurements and Calculations

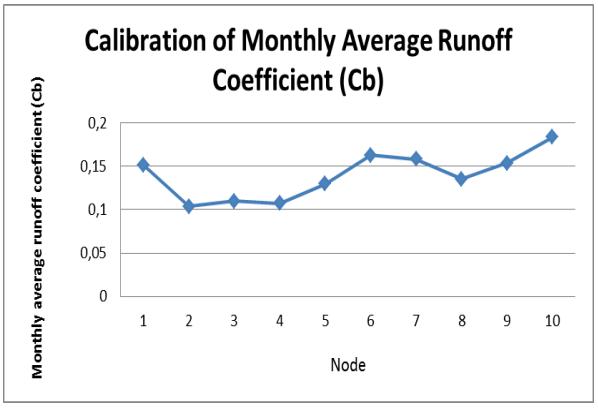

Figure 2. Calibration of Monthly Average Runoff Coefficient (Cb) on October 2010

The pattern of monthly average runoff coefficient (Cb) from point 1 to point 2 decreased, then from point 2 to point 3 there was further increase in the runoff coefficient of the monthly average (Cb) slightly down to the point 4 and started to increase again at the point 5. From point 5 to point of the monthly average 6 runoff coefficient (Cb) increased again and reached a peak further decreased at point 7 to point 8, and the monthly average runoff coefficient (Cb) increased back at point 9. From the above discharge pattern can be seen that the runoff coefficient of the monthly average (Cb) obtained ranged from 0.10 to 0:18 with an average value of 0.1394. This value represents the initial conditions of the rainy season with the amount of rainfall in October 2010 amounted to 318 mm / month that occurred during the 25 days of rain. The smaller the value of the monthly average runoff coefficient (Cb) shows the less rainfall runoff cause infiltration into the ground more and the greater of average monthly runoff coefficient (Cb) show more runoff to infiltrated into the ground. This was consistent with the statement of Suripin (2004) that the infiltration rate decreased in conditions of continuous rain [2]. This was because the soil has been saturated water so that rain water and the less water infiltrated rain water runoff becomes more.

Reaction Against calibration coefficient (K)

Calibration of the reaction coefficient was done by adjusting the concentration of nitrate in the field of measurement results with the results of calculations by the nitrate concentration in the model so that get a reaction coefficient (K). Nitrate concentration data in the field measurement results are presented in Figure 3.

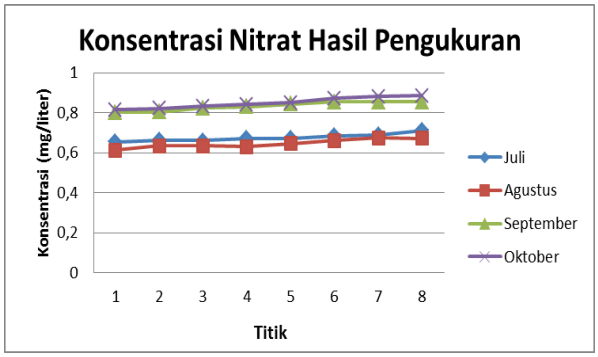

Figure 3 Measurement Nitrate Concentration

Figure 3 it can be seen that the nitrate concentrations occur in Rembangan river Jember in July 2010 to October 2010 Month pattern has increased from the upstream to the downstream. The concentration of nitrate in July 2010 and August 2010 have a value with an interval of about 0.6 mg / liter to 0.7 mg / liter and the concentration of nitrate was lower than in September 2010 and October 2010. July 2010 and August 2010 was the condition before fertilization so that the concentration of nitrate in river water has not been affected too much by the addition of nitrate compounds from the coffee plantation.
While the concentration of nitrate in September 2010 and October 2010 also has an interval value of about 0.8 mg / liter to approach the value of 0.9 mg / liter with nitrate concentration value greater than in July and August 2010 because it was the condition after fertilization in the land coffee plantations so that the concentration of nitrate in river water has been affected by the addition of nitrate compounds from the coffee plantation.

The results of the calibration coefficient (K) was presented in Figure 4 and Figure 5 below.

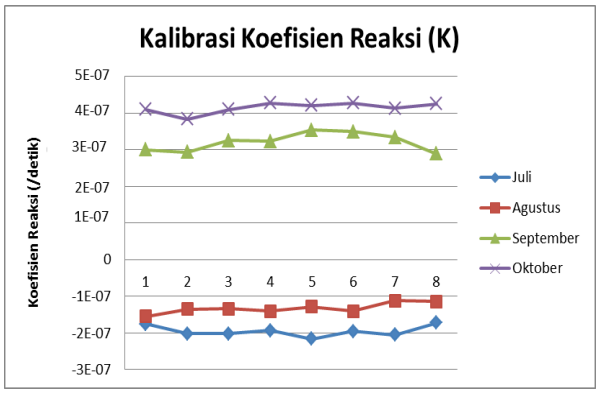

Figure 4. Fluctuation Calibration Results Reaction Coefficient (K) at each point of observation.

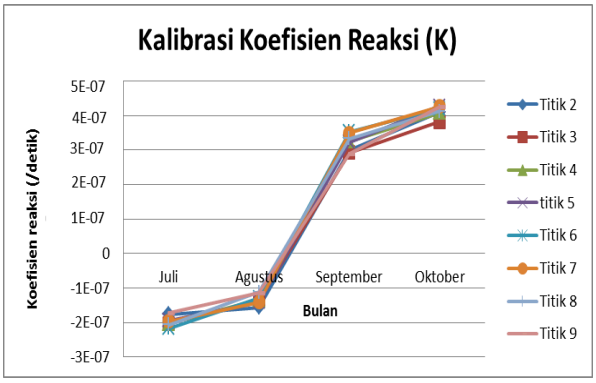

Figure 5. Fluctuations Calibration Results Reaction Coefficient (K) Every Month.

From the calibration process in Figure 4 Results calibration reaction coefficient (K) was the difference between the results of the calibration pattern reaction coefficient in the period July 2010 and 2010 with the results of the calibration Agusutus reaction coefficient in the period September 2010 and October 2010 in the period July 2010 and August 2010 showed the value of the reaction coefficient of -1.6395 x 10-6 / sec with a minus sign , which means a decrease or reduction in the rate of reaction on the Rembangan river because the month was the month that represents the dry season with rainfall fairly small. Meanwhile, in September 2010 and August showed a reaction coefficient of 3.6775 x 10-6 / sec , which means an increase in the reaction rate for the month is the rainy season and the addition of nitrogen into the water stream as a result of fertilization is done on Rembangan coffee plantations Renteng Jember so on to increase the amount of river water nitrate concentrations of compounds .

Table 2. Concentrations of Nitrate Model Calibration Results.

Node	Juli 2010		Agustus 2010		September 2010		Oktober 2010	
	Nitrate concen tration (mg/ltr)	Calibration of K	Nitrate concen tration (mg/ltr)	Calibration of K	Nitrate concen tration Nitrat (mg/ltr)	Calibration of K	Nitrate concen tration (mg/ltr)	Calibration of K
2	0.6549	-1.759 x 10^{-6}	0.612	-1.552 x 10^{-6}	0.8008	3.004 x 10^{-6}	0.814	4.097 x 10^{-6}
3	0.6635	-2.021 x 10^{-6}	0.6339	-1.353 x 10^{-6}	0.8045	2.931 x 10^{-6}	0.8201	3.830 x 10^{-6}
4	0.66	-2.024 x 10^{-6}	0.6339	-1.344 x 10^{-6}	0.8233	3.255 x 10^{-6}	0.8323	4.094 x 10^{-6}
5	0.6712	-1.933 x 10^{-6}	0.6311	-1.403 x 10^{-6}	0.8308	3.233 x 10^{-6}	0.8416	4.276 x 10^{-6}
6	0.6712	-2.164 x 10^{-6}	0.6448	-1.279 x 10^{-6}	0.8421	3.535 x 10^{-6}	0.8506	4.207 x 10^{-6}
7	0.6848	-1.956 x 10^{-6}	0.6612	-1.401 x 10^{-6}	0.8534	3.498 x 10^{-6}	0.872	4.270 x 10^{-6}
8	0.6902	-2.057 x 10^{-6}	0.6749	-1.121 x 10^{-6}	0.8534	3.336 x 10^{-6}	0.8811	4.134 x 10^{-6}
9	0.712	-1.726 x 10^{-6}	0.672	-1.138 x 10^{-6}	0.8534	2.887 x 10^{-6}	0.8841	4.250 x 10^{-6}
Average	0.676013	-1.955 x 10^{-6}	0.6455	-1.324 x 10^{-6}	0.832713	3.210 x 10^{-6}	0.84955	4.145 x 10^{-6}

Source : Results of Field Measurements and Model Calculations.

It can be concluded that the Rembangan river will decrease or reduction in the rate of reaction of nitrate compounds in the dry season with little rainfall and will increase the reaction rate or the addition of nitrate compounds in the conditions of the rainy season with rainfall greater.

4. Conclusion

Based on the above it can be concluded as follows :

1. The results of the calibration coefficients of the monthly average runoff (Cb) in October 2010 showed a similar pattern with the data flow graph that is increasing fluctuation in the value of coefficient of runoff downstream with an average of 0.1394.
2. The results of the calibration coefficient of reaction (K) was the difference between the results of the calibration pattern reaction coefficient in the period July 2010 and Agusutus 2010 with the results of the calibration coefficient of the reaction in the period September 2010 and October 2010 in the period July 2010 and August 2010 showed reaction coefficient of -1.6395 x 10-6 / sec with a minus sign , which means a decrease or reduction in the rate of reaction on the river Rembangan . Meanwhile, in September 2010 and August showed

a reaction coefficient of 3.6775 x 10-6 / sec , which means an increase in the reaction rate .

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*) *the reference which is not cited in the text can be considered for further reading or bibliography*

The Changes of Phosphate Fraction in Andisol and Oxisol by Organic Matter Application

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Abstract **-** Soil phosphate (P) exists in various chemical forms, including inorganic P (Pi) and organic P (Po), which differ widely in their behavior and fate in soils. Some fractions of soil P are insoluble forms and each concentration depends on soil types. The problem of P deficiency in Andisol and Oxisol can potentially be solved by the progressive return of organic materials to soil. However, the quantity, quality, and management of this practice are fundamental factors that affect the availability of P from residues. The objectives of this research are to evaluate: (1) the changes of fractions of soil P in Andisol and Oxisol by adding organic acid and organic matter, and (2) enhancement of available soil P in Andisol and Oxisol. Four treatments have been done i.e: (1) control, (2) citric acid, (3) oxalic acid, and (4) soybean biomass. Soil was incubated for 30 days. The results show that application of soybean biomass increases the total P–and the availability of P in both soil types. Furthermore, model of changes of soil P fractionation are different between Andisol and Oxisol. In addition, 30 days incubation does not influence the decreased of P retension in Andisol; it shows that adsorption site of Andisol is higher than Oxisol.
Key word: Andisol, Oxisol, soil phosphate fraction

1. Introduction

Phosphorus is an essential nutrient. In spite of its wide distribution in nature, P is a limited resource and it is deficient in most soils. Soil P exists in various chemical forms, including inorganic P (Pi) and organic P (Po), which differ widely in their behavior and fate in soils, specifically in relation to bioavailability, as various forms can undergo cycling at different rates, being retained in soils or made available to plants.

In general, Andisols are predominated by amorphous materials such as Al-oxides, Fe-oxides, allophane, and imogolite. These soils have a high P-sorption capacity due to the very high surface area of allophanic, amorphous minerals, and Humus-Fe complexes [1]. Andisol has a high P-sorption capacity about 8000 – 15000 ppm [2]. However in acid soil such as Oxisol and Ultisol, orthophosphate anion will react with Al and Fe fraction to create undisolve $AlPO_4$ and $FePO_4$ (precipitated).

The variable charge mineral constituents (≡Al-OH and ≡Fe-OH) have an important role in adsorbing some oxyanions. Due to their high surface area and reactivity, they strongly retain anions such as phosphate, borate, carboxylate acids (acetate, oxalate, citrate), molibdate, silicate, sulphate, and organic matter and either as adsorbed species or as a reaction products. The principal sorption mechanism is ligand exchange involving the formation of inner-sphere surface complexes.

Fractionation of P in soil influences the availability of P. The occurrence of such fraction is affected by the presence of other anions that able to bind with metal and compete with phosphate, among others are organic anions. These anions could be produced by microbes and roots activities as well as decomposition of organic materials.

Organic materials not only serve as source of macro and micro nutrients for plants, but also closely related to the population and the activities of soil biology. Organic acids, as results of the decomposition of organic materials, have an ability to bind cations through chelation bond. They are also able to envelop the positively charged colloidal. Returns of crop residues into the soil give the change in the P-labile, total P-organic, and P fractions. Moreover, such a process is able to extract the increase in P. However, [3]Dahgreen and Drisscoll (1994) reported that the change of H_2PO_4 concentration in the soil solution is not real. It seems that the change of P in the soil, after the addition of organic matter, is inconsistent. The change of P could be influenced by several factors, among others: the level of addition of materials, the quality of materials, the analyzed P, the status of P in the soil, and the characteristics of each soil type.

Generally, different soil characteristics lead to the different pattern of its fractionation. The addition of organic matter will affect the pattern of fractionation, but how big and how the pattern changes are still not much investigated yet until now.

Organic ligands such as tartaric acid, oxalate, malic and citric containing carboxyl group (COOH), aliphatic phenolic-OH,-hydroksil are very effective in

dissolving minerals and the formation of the elements Al, chelat Fe, Ca as well as other elements and lowers the pH of the medium [4].

The effect of carboxylic groups (e.g. citric, malic, oxalate) as an exudates component generated by root or microbes is able to mobilize the form of P-organic (Po) and the P-inorganic (Pi) due to the formation of metal-cation complexes that bind and move P from soil matrix with ligand exchange.

This study was set up to evaluate the changes of fractions of soil P and enhancement of available soil P in Andisol and Oxisol by adding organic acid and organic matter. Results of the study will assist in the development of long-term P management strategies for improved soil fertility especially in soil phosphate status.

2. Method

Research was conducted at Soil chemistry Laboratory Faculty of Agriculture University of Jember. Research was done by factorial Randomized Complete design (three replicate) with the following factors.

Factor-1: Soil type: 1. Andisol (from Batu East Java)
2. Oxisol (from Depok West Java)

Factor-2: Organic compound: 1. Control;
2. Citric acid (1M); 3. Oxalic acid (1M)
4. Organic matter (soybean biomass)

2.1 Material Preparation

Andisol samples were taken from Batu East Java, while Oxisol samples were taken from Depok West Java. Samples were collected at the <15 cm depth only. Soil samples were placed in plastic bags. Soils were air-dried and then sieved through a 2mm mesh screen. Approximately 5000 g.pot^{-1} of each soil was prepared as medium with 80% field capacity condition. Initial analysis was soil pH, texture, organic carbon, total and available phosphate, Nitrogen total and fractionation of phosphate. Artificial citric and oxalic acid are applied to compare the effectiveness of increasing the availability of phosphate and soybean biomass. Shoot of soybean biomass was decomposed naturally about thirty days. Then the results were analysed on organic acid (citric, oxalic, fumaric, acetic acid).

2.2 Application

Each soil types added by 100 ml.pot^{-1} organic acid for organic acid treatment, and 70 g soybean biomass was incubated for thirty days on 80% field capacity conditions. Analysis of pH, total-P (HCL 25%) and available-P (Bray) was done regularly (every ten days), while fractionation-P (Hesse, 1972) was measured at the end of incubation.

2.3 Material Preparation

Andisol samples were taken from Batu East Java, while Oxisol samples were taken from Depok West Java. Samples were collected at the <15 cm depth only. Soil samples were placed in plastic bags. Soils were air-dried and then sieved through a 2mm mesh screen. Approximately 5000 g.pot^{-1} of each soil was prepared as medium with 80% field capacity condition. Initial analysis was soil pH, texture, organic carbon, total and available phosphate, Nitrogen total and fractionation of phosphate. Using artificial citric and oxalic acid are compare the effectiveness increasing availability of phosphate with soybean biomass.

Shoot of soybean biomass was decomposed naturally about thirty days. After that, these product of decomposition were analysed on organic acid (citric, oxalic, fumaric, acetic acid).

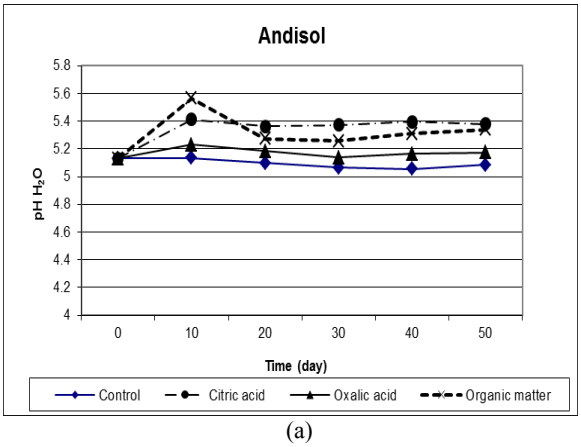
Application

In each soil added by organic acid (100 ml.pot^{-1}) and 70 g biomass. Incubate for thirty days on 80% field capacity conditions. Analysis of pH, total-P (HCL 25%) and available-P (Bray) was done regularly (every ten days), and fractionation-P (Hesse, 1972) at the end of incubation.

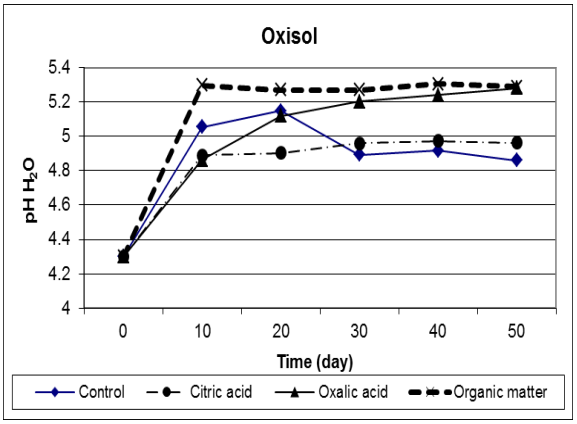
3. Result and Discussion

3.1 Soil Reaction (pH)

The addition of artificial organic acid and organic matter (soybean biomass) changes the soil acidity on both soil types as shown by Figures 1a and 1b. It can be seen from those figures that in Oxisol, all treatment increased soil pH. In contrast, the increase of soil pH on Andisol was only taken place till D-10 and then subsequently decreased. It can be observed that for Andisol, soil pH was higher than the initial soil pH except for control.



(a)



(b)

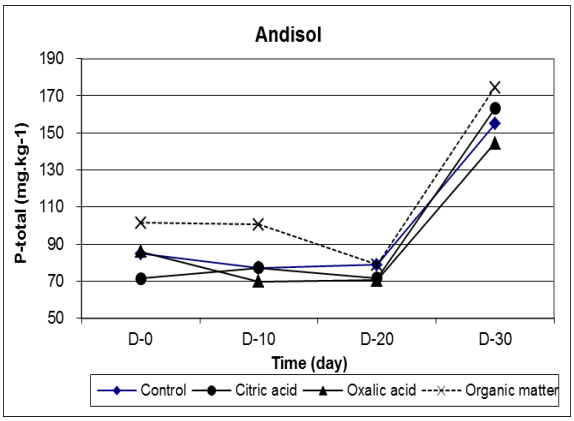
Figure 1. Changes of pH on Andisol (a) and Oxisol (b)

The increase of pH occured due to ionization of functional groups of organic matters, i.e carboxyl (-COOH), phenol (-OH), enol (-OH), quinon and amide [5] that causes high phenolic groups concentrations in the soil solution, leading to the increasing soil pH. In addition, organic matter has ability as a *buffering capacity* of soil pH by binding or release H^+ ions, resulting in a pH close to neutral [6] .

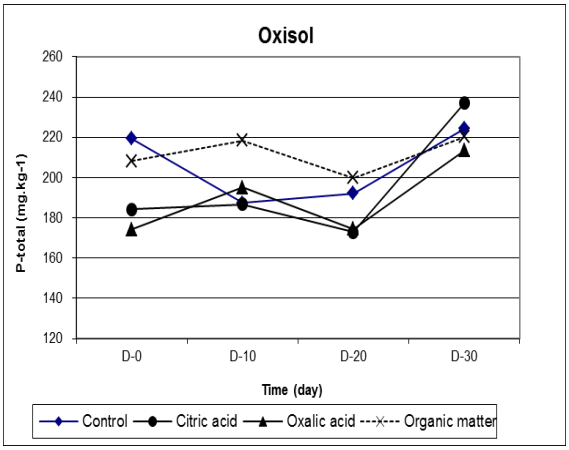
3.2 P-total (HCl 25%) and P-available (Bray II)

Figures 2a and 2b shown the behaviour of P-total (HCl 25%) in Andisol and Oxisol for different treatments. It can be seen that both soil types had similar trend, i.e. P-total was tend to decrease until D-20, and then sharply increased after D-20. Compared to other treatments, the addition of organic materials resulted in highest concentration of P-total.

The addition of organic materials also produced highest P-available soil (Figures 3a and 3b). The decomposition of organic material will increase P-organic form such as phospholipida, nucleic acids and inositol phosphates.



(a)

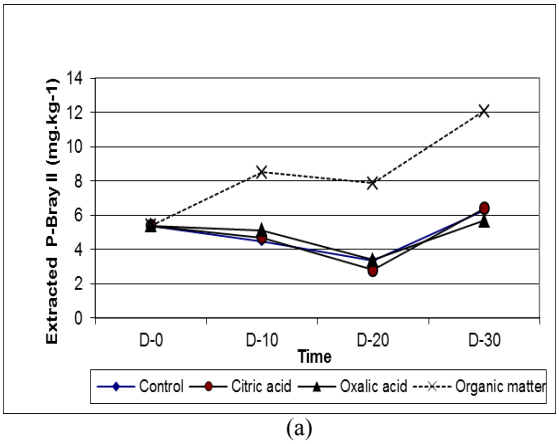


(b)

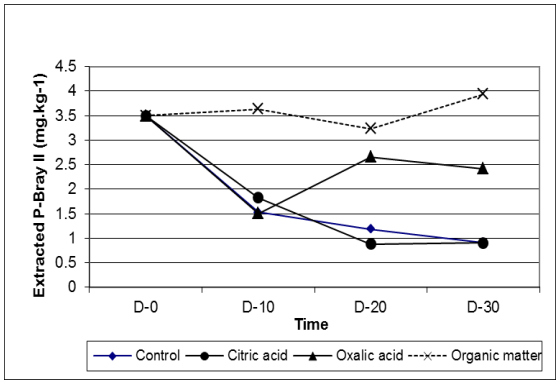
Figure 2. Changes of P-total (HCl) on Andisol (a) and Oxisol (b)

The changes of P-available (Bray II) by addition of organic matter was due to the process of mineralized organic-P to P-inorganic form. Phosphate mineralized from soybean biomass occured due to the ratio of C/P is less than 200. Based on initial analysis, C/P of soybean biomass was 102. The changes also took place due to the competition of adsorption site between organic anion and orthophosphate anion. If the concentration of organic anion in soil solution was higher than orthophosphate anion, it will replace the orthophosphate anion in adsorption site, so the concentration of the orthophosphate anion in the soil solution increased.

It can also be observed from Figures 3a and 3b that the effect of additional organic matter to the increase of concentration of soil P-available in Andisol was larger than in Oxisol for the whole periode of incubation. Decomposition of organic matter produces organic acid continuously, so that the concentration of organic anions that compete with phosphate anions in soil was much bigger. It could produce metal-organic forms by chelation mechanisms.



(a)



(b)

Figure 3. Changes of P-available (Bray II) in Andisol (a) and Oxisol (b)

Andisol formed from the ashes of Vulcan materials with the dominance of amorphous materials (allophane, imogolite and humus fraction) that has a large specific surface area, so adsorption from Adisol is also great. The large P retention on Andisol cause release P from adsorption site of allophane and organic matter. Research results [7] on the Andisol soil treated with the addition of fertilizer P increases the concentration of P-in organic, but not P-organic.

The existence of a large P retention in Andisol also led to a release of P from the sorption of allophane and organic matter. Andisol formed from volcanic ash materials with the dominance of amorphous materials (allophane, imogolit and humus fraction) having an extensive specific surface. Early research [7] reported that in Andisol treated with the addition of P fertilizer increased the concentration of P in organic, but not P-organic.

3.3 The Changes of P Fraction

The pattern of the change in the fraction of P in Andisol and Oxisol was significantly different as shown in Tables 2 and 3. The data in Table shown that in Oxisol, total concentration of P fraction for all soil types decreased compared to control soil. In contrast, total concentration of P fraction in Andisol increased compare with control soil.

The increase of total fraction in Andisol was higher than in Oxisol. Such a result is due to Andisol has large adsorption capacity. P retention in Andisol before treatment was 94.9%, while P retention in Oxisol was only 60.3%. After 1 month incubated, adsorption site of Andisol was still high, consequently the amount of total P fraction increased over control.

Table 2. The changes of soil P-fraction concentration at 30 days

Treatment	Soluble - P	Al-P	Fe-P	Ca-P	Red-P	Occ-P	total
	mg.kg^{-1}						
Andisol							
Control	0.54	0.70	-	5.18	5.97	2.83	15.21
Citric acid	0.35	1.43	-	5.35	10.47	4.53	22.13
Oxalic acid	0.67	0.81	-	5.45	15.70	3.82	26.44
Organic matter	0.67	0.88	-	5.55	23.27	3.19	33.56
Oxisol							
Control	0.70	0.40	12.69	4.15	3.33	2.99	24.27
Citric acid	0.37	1.36	7.05	3.85	5.88	2.39	20.89
Oxalic acid	1.03	0.81	7.79	3.80	4.16	2.00	19.59
Organic matter	0.39	0.70	10.29	5.68	1.67	1.34	20.05

Addition of citric acid, oxalic acid, and organic materials had different results in the changes of P-fraction in Oxisol as well as in Andisol. In Oxisol, organic matter decreased all of P-fraction except Al-P and Ca-P, but in Andisol all P-fraction increased having added by organic matter. Basically organic material has the ability to release P into the soil solution, but the organic material itself has component which able to serve as new adsorption site, so that P that has been released can be adsorped again.

Conversely, the artificial organic acid (citric and oxalic acid) do not have adsoption site which can adsorp or discharge of P, only dissociation of organic anion. Then anion organic compete with orthophosphate ion to build organic-anion complexes.

From Table 3, it is observed that organic matter significantly decreased undissolve phosphate in Oxisol i.e occluded-P, reductan-P and Fe-P. It dominated fix by iron or Fe-oxide. In Oxisol, ferrous oxide was high, as recorded in initial data (appendix 1), ratio of Feo/Fed was low (0.23 - 0.38).

Table 3. Percentage of soil P-fraction at 30 days

Treatment	Soluble – P	Al-P	Fe-P	Ca-P	Red-P	Occ-P	total
	%						
Andisol							
Control	3.57	4.58	-	34.03	39.24	18.59	100.00
Citric acid	2.30	9.40	-	35.18	68.83	29.81	145.52
Oxalic acid	4.37	5.30	-	35.84	103.24	25.10	173.85
Organic matter	4.37	5.79	-	36.50	152.99	21.00	220.65
Oxisol							
Control	2.88	1.66	52.30	17.10	13.73	12.32	100.00

Citric acid	1.51	5.59	29.04	15.86	24.24	9.83	86.08
Oxalic acid	4.25	3.32	32.10	15.66	17.13	8.23	80.70
Organic matter	1.59	2.87	42.41	23.38	6.87	5.50	82.62

High organic matter concentration in Andisol affected P-fractionation model. Andisol has big capability to adsorp phosphate, strong and very slow ability to desorp; as a result, percentage of P was still large until D-30. However, P fixation in Oxisol was weak, so that P could be changed to other P form. Organic matter from soybean biomass significantly decreased insoluble P fraction in Oxisol such as occluded-P, reductan-P and Fe-P. Previous research by [8] shown that Andisol has higher capacity to P adsorb than Latosol. In addition the adsorption curve in Andisol linearly increased until 3 months incubated.

4. Conclusion

1. The changes of P fraction in Andisol and Oxisol have different patterns.
2. In Andisol all P-fraction increase, except for soluble-P, but in Oxisol the treatments decrease Fe-P, reductan-P and occluded-P.
3. All of treatments can increase the availability of P concentration, at which the addition of organic matter serves the biggest effect.

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