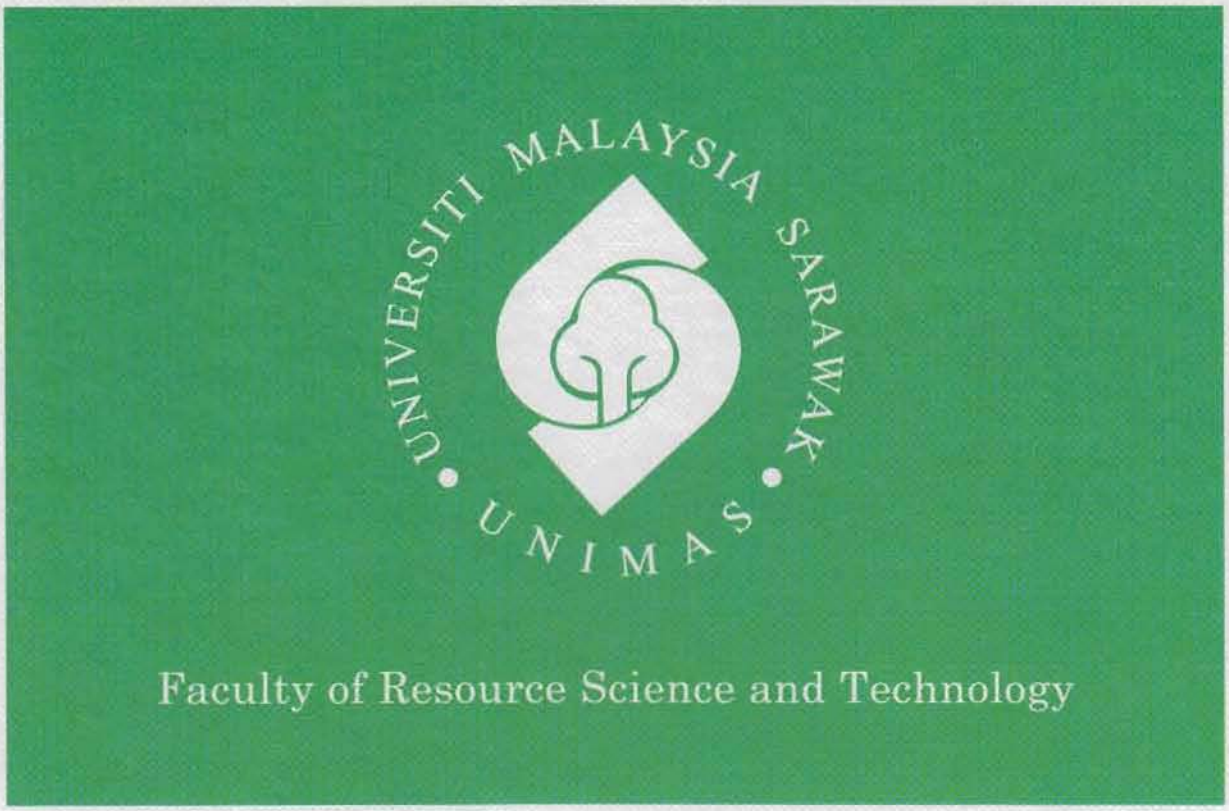

PRELIMINARY STUDY ON SAGO FROND JUICE AS AN INOCULUM MEDIA FOR THE GROWTH OF *Lactococcus lactis* I0-1

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(45606)

Bachelor of Science with Honours
(Resource Biotechnology)
2017

Preliminary Study on Sago Frond Juice as an Inoculum Media for the Growth of *Lactococcus lactis* I0-1

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(45606)

This project is submitted in partial requirement for the degree of
Bachelor Science with Honours
(Resource Biotechnology)

Supervisor: Dr Dayang Salwani Awg Adeni
Co-Supervisor: Prof. Dr. Kopli Bujang

Resource Biotechnology Programme
Faculty of Resource Science and Technology
Universiti Malaysia Sarawak
2017

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I **Iwana Haziqah Binti Mohd Taufik (45606)** from **Faculty of Resource Science and Technology** hereby declare that the work entitled **Preliminary Study on Sago Frond Juice as an Inoculum Media for the Growth of *Lactococcus lactis* I0-1** is based on my original work, except for the quotations and citations which have been dully acknowledged also, declare that it has not been or concurrently submitted for any other degree at UNIMAS or other institution of higher learning.

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ACKNOWLEDGEMENT

Bismillahirrahmanirrahim. In the name of Allah Taala, The Most Gracious, Most Merciful Shalawat and remembrance for the Holy Prophet, Muhammad S.A.W.

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LIST OF ABBRIEVIATIONS

^{O}C	Degree Celsius
DCW	Dry cell weight
DNS	Dinitrosalicylic acid
OD	Optical density
g/L	Gram per litre
kg	Kilogram
mL	Millilitres
rpm	Revolution per minute
CG	Commercial glucose
SFJ	Sago frond juice
ha	Hectare
h	Hour
nm	Nanometer

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Preliminary Study on Sago Frond Juice as an Inoculum Media for the Growth of *Lactococcus lactis* I0-1

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ABSTRACT

This research was conducted to study the ability of sago frond juice (SFJ) as an inoculum media for growing *Lactococcus lactis* I0-1. *L. lactis* I0-1 is lactic acid producing bacteria which commonly consumes glucose as its carbon source. The bacteria was incubated in four varieties of inoculum media which were SFJ only, SFJ with 2.5 g/L yeast extract, SFJ with 5 g/L yeast extract whereas commercial glucose (CG) with 5 g/L yeast extract was used as control throughout this study. From the result, SFJ with 5 g/L yeast extract and CG with 5 g/L yeast extract exhibited almost similar maximum DCW of *Lactococcus lactis* I0-1. However, in terms of time, the *Lactococcus lactis* I0-1 in CG with 5 g/L yeast extract was faster to reach maximum DCW compared to SFJ with 5 g/L yeast extract.

Keywords: Sago frond juice, *Lactococcus lactis* I0-1, inoculum, commercial glucose

ABSTRAK

Kajian ini dijalankan untuk mengkaji keupayaan jus pelepah sagu (SFJ) sebagai media inokulum untuk pertumbuhan Lactococcus lactis *I0-1.* Lactococcus lactis *I0-1 adalah bakteria yang menghasilkan asid laktik yang kebiasaannya menggunakan glukosa sebagai sumber karbon. Bakteria* Lactococcus lactis *I0-1 telah diinkubasi di dalam 4 jenis media inokulum iaitu SFJ sahaja, SFJ bersama 2.5 g/L ekstrak yis, SFJ bersama 5 g/L ekstrak yis manakala glukosa komersial (CG) bersama 5 g/L ekstrak yis telah digunakan sebagai kawalan sepanjang kajian ini. Kajian ini mendapati SFJ bersama 5 g/L ekstrak yis dan CG bersama 5 g/L ekstrak yis menghasilkan maksimum DCW* Lactococcus lactis *I0-1 yang hampir sama. Namun begitu, dari segi masa,* Lactococcus lactis *I0-1 di dalam CG bersama 5 g/L ekstrak yis lebih cepat untuk mencapai maksimum DCW berbanding SFJ bersama 5 g/L ekstrak yis.*

Kata Kunci: *Jus pelepah sago,* Lactococcus lactis *I0-1, inokulum, glukosa komersial*

1. INTRODUCTION

In sago plantations, the industry usually produces commercial starch that is derived from the stem of sago palm (Karim *et al.,* 2008). Ishizuka *et al.* (1995) stated that a large amount of starch is found in the sago palm's trunk and its productivity was calculated to be 4 times that of paddy rice. Due to this factor, sago is mostly known for its starch production. Nevertheless, other parts of sago plant also are able to produce product. One of the parts is the sago frond which is the basal part of the sago leaves. During harvesting and pruning, thousands of sago fronds are disposed per day (Bujang, 2006). The fronds will be left out at the plantation floor mainly for maintaining the nutrients in the soil purposes (Che Maail *et al.*, 2014). However, the downside of this action is it can create environmental pollution due to the slow degradation of the frond. Thus, the alternative way to reduce this problem is by utilizing the frond for various value-added products.

Sago frond is able to produce sago frond juice (SFJ) that has the potential to be used as inoculum media or fermentation feedstock as it is discover to contain free sugar. Other studies on utilizing frond using oil palm frond (OPF) juice had been conducted by several researchers revealed that fermentation of OPF juice is comparable with using technical grade sugars (Zahari *et al.*, 2012). A study by Lim *et al.* (2012) showed that the fermentable sugar obtained from OPF could be used by *Saccharomyces cerevisiae* to produce ethanol about 23.1 g/L. OPF juice also could be used in fermentation of succinic acid as proved by Tan *et al.* (2016) where fermentation of OPF with *Actinobacillus succinogenes* 130Z gave a final concentration of succinic acid production up to 21 g/L after 60 hours of anaerobic fermentation. This study will emphasize on SFJ as inoculum

media to grow *Lactococcus lactis* I0-1, which bacteria that is commonly used in fermentation of lactic acid.

Therefore, the aims of this study are to:-

1. To utilize the sago frond juice as inoculum media for growing *Lactococcus lactis* I0-1 bacteria.
2. To determine the growth profile of *Lactococcus lactis* I0-1 bacteria in sago frond juice as a sole inoculum media and SFJ amended with yeast extract.

2. LITERATURE REVIEW

2.1 Characterization of Sago Palm

2.1.1 Sago Palm Plantation

Sago palm (*Metroxylon sagu*) plantations are majorly found in the state of Sarawak which comprises more than 90% in Malaysia (Bujang, 2014). The largest sago planting is located in Mukah which covers 75% of Sarawak's total land area, where over 50% of the sago starch is produced (Bujang, 2014). Studies on sago palm started to get more attention in the 1970s, where export of sago flour was fast becoming one of the important agricultural export commodities at that time (Karim *et al.*, 2008). 28000 metric tons of industrial grade sago starch was exported with earning about RM3.8 million in 1970 to about RM8.8 million for about 26000 metric tons in 1980. Malaysia Department of Statistic stated that in 2005, export of about 45300 metric tons of food-grade sago starch earned about RM40.4 million. The current price of sago starch is now at an all times high RM1800/ton and Sarawak exported about 60, 000 tons in 2011, earned about RM91.04 million (Bujang, 2014).

2.1.2 Properties of Sago Leaves and Fronds

A single sago palm tree has between 7-24 fronds (Craun Research, 2014). The length of the frond is between 5-8 m and each of the fronds carries 100-200 leaflets (Hirst, 2014). Length of the leaves is from 72-203 cm with single large and hard midrib.

2.2 Sugar Industry

2.2.1 Potential of Biomass to Produce Sugar

Biomass from plant is rich in sugar and also can be as an alternative to petrochemicals (Guerriero *et al.*, 2016). Drechsel & Kunze (2002) stated that plant biomass can be recycled to be used in other manufacturer sector. The sugar from that derived from the biomass can be fermented and produce bioethanol. In addition, bioethanol from biomass can be as an alternative to reduce both environmental pollution and the consumption of crude oil (Demirbas, 2003).

2.2.2 Production of sugar from sago frond

Sago palm industry in Malaysia is concentrated mostly in the river areas in Sarawak, which covers an area of 1.5 million ha, about 12% of Sarawak's total land area (Tie & Lim, 1977) and is exploited as both staple and a cash crop in Sarawak. Currently, sago fronds are left rotting between the sago palm trees, mainly for soil and nutrients conservation and also erosion control (Hassan *et al.*, 1995). Several studies has been done using other frond such as oil palm frond, to make effective utilization of the frond, however, using sago frond is not yet being conducted. Based on the study on oil palm frond, sago frond also has the potential to be utilized and be used in various areas. It was found that sago frond also contain high sugar concentration. To extract the sugar or glucose, just simply squeezed the frond and the raw sugar is obtained.

2.3 Lactic Acid Production

2.3.1 Lactic Acid Bacteria

The first pure of lactic acid bacterium ("*Bacterium lactis*") was discovered by J. Lister (Konig & Frohlich, 2009). A typical lactic acid bacterium grown in unlimited glucose concentration, oxygen limitation and growth factors or also known as the standard conditions is nonsporing, gram positive, aerotolerant, catalase negative in the absence of porphorinoids, acid tolerant, organotrophic and a strictly fermentative rod or coccus, which produce lactic acid as the main end product. Lactic acid also lacks of cytochromes and is unable to synthesize porphyrins. Besides, lactic acid bacteria can produce variety of antagonistic factors that include metabolic-like substances and antimicrobial proteins or bacteriocins (Klaenhammer, 1988; Lindgren & Dobrogosz, 1990). However, its features may vary under certain conditions.

2.3.2 Inoculum Preparation for Lactic Acid Bacteria

According to Stanbury *et al.* (1995), all microorganism require sources of energy, water, nitrogen sources, carbon, mineral elements, and if aerobic vitamins plus oxygen. Substrates such as glucose, starch, sucrose, and lactose as carbon sources and nitrates, ammonium salts, and urea as nitrogen sources is commonly used for fermentation process as it is able to produce high yield of product or biomass and also have consistent quality. Lactic acid bacteria compose of ecologically group of microorganisms unified by formation of lactic acid as the primary metabolite of sugar metabolism (Carr *et al.*, 2002; Liu, 2002). Its utilize sugars by either hetero- or homo- fermentative pathways. These bacteria are described as nutritionally fastidious bacteria due to have very limited biosynthetic

capabilities (Fugelsang & Edwards, 2007). Early work done by Du Plessis in 1963, reported that all strains of wine lactic acid bacteria required riboflavin, panthothenic acid, nicotinic acid, and either pyridoxine or thiamine for growth. Several amino acids such as valine, arginine, glutamic acid, isoleucine and leucine also appear to be necessary for the bacteria growth. Other than that, study by Garvie and Mabbitt (1967), proved that another important nutrient required by lactic acid bacteria is the so-called tomato juice factor. This is due to the fact that many lactic acid bacteria seemed to grow on media supplemented with either fruit or vegetables serums or juices. Therefore, SFJ was used in this study to determine its capabilities as the inoculum media for lactic acid bacteria.

2.3.3 Fermentation of Lactic Acid

Fermentation process that produce lactic acid is depict as a major green chemical product as this process does not exacerbate the level of greenhouse gases particularly carbon dioxide (Ishizaki, 1997). Moreover, Bujang *et al.* (2004) stated that L-lactate is the precursor in the synthesis of polylactate (PLA), which is a prerequisite bioplastic material. PLA belongs to the family of alphatic polyesters usually made from α-hydroxy acids, which include polyglycolic acid or polymandelic acid. It is a high-strength, high modulus polymer, and thermoplastic that can be made from annually renewable resources (Garlotta, 2002). Application of lactic acid creates the emergence of new markets such as biodegradable thermoplastics together with the more traditional industries such as pharmaceuticals, tanning of leather, food and beverages and also cosmetics. In addition, lactate also is widely used in various industrial applications including chemical, detergents, textile, electronics and metal (Bujang *et al.*, 2001). This event has created an impetus for the lactate industries to develop into a larger scale.

2.3.4 Production of Lactic Acid from Selected Agricultural Source

Lignocellulosic biomass was used for the production of lactic acid as an alternative raw material recently. Lignocellulose is composed of cellulose and hemicellulose that is made up of hexose and pentose sugar surrounded in phenolic polymer lignin matrix. The main procedure depends on enzymatic hydrolysis by cellulolytic and hemicellulolytic enzymes to produce sugars from lignocelluloses which are fermentable. A pretreatment, either chemical or mechanical is required of the lignocellulose to reduce the size of particle, to remove the lignin or to modify it and to improve convertibility of the polysaccharides for the purpose of enzymatic hydrolysis (Maas *et al.*, 2008).

Several studies have been done using selected agricultural sources in fermentation lactic acid. A study by Timbuntam *et al.* (2006), noted that fermentation of sugarcane juice as carbon source for the production of lactic acid was homofermentative with 90% yield after 10 hours of fermentation under controlled pH condition. Five strains of lactic acid bacteria were used in the experiment which was *Lactobacillus plantarum* SP1-3, *Lb. pentosus* KUB-ST 10-1, *Lb. salivarius* ssp., salivarius TISTR 1112, *Lb. delbrueckii* ssp. *bulgaricus* TISTR 895 and *Lb. casei* TISTR 390. *LB. casei* TISTR 390 shows the highest lactic acid production from the fermentation of sugar cane juice. Sago frond juice also was used as raw material for the production of lactic acid via fermentation of *Lactococcus lactis* I0-1 as producer of pure L-lactate. The fermentation was controlled under optimum growth condition at pH 6 and at 32 °C that help to enhance the growth of *Lactococcus lactis* I0-1 and produce higher concentration of lactate (Bujang *et al.*, 2005).

2.4 Importance of inoculum for fermentation

Different fermentation process requires different media in order to grow. Therefore, to obtain the optimum medium for an individual fermentation process a thorough investigation is needed. However, certain basic requirements must be met by any such medium. By doing inoculum preparation, it can provide a suitable environment for the microbe before they perform in real fermentation. Besides that, a proper inoculum preparation can save lag phase of the microbe during real fermentation. The sterility of the microbe also can be checked trough inoculum preparation to detect contamination.

3. MATERIAL AND METHODS

3.1 Materials

3.1.1 Fresh sago frond

Fresh sago frond (**Figure 3.1**) was obtained from Paya Paloh, Kota Samarahan, Sarawak. The basal part of the sago frond petiole was skinned off and weighted.

Figure 3.1 Fresh sago fronds. (A) Different size of sago frond. (B) The sago frond was weighted. (C) The skinned off sago frond. (D) Fibre of sago frond.

3.1.2 Commercial glucose

The commercial glucose (**Figure 3.2**) that used as control was obtained from Biochemistry Laboratory of Faculty Resource Science and Technology.

Figure 3.2 Commercial glucose.

3.1.3 Bacteria

Lactococcus lactis I0-1 used in the fermentation was obtained from the stock culture (**Figure 3.3**) which was kept at 4 °C at Biochemistry Laboratory of Faculty Resource Science and Technology.

Figure 3.3 Stock culture of *Lactococcus lactis* I0-1.

3.2 Methods

For all experiments and analysis, an average of two trials was used as analytical data. The inoculum preparation from fermentation process of *Lactococcus lactis* I0-1 using commercial glucose also was conducted as the control treatment.

3.2.1 Sago frond Juice Preparation and Extraction

Approximate 2 to 5 kg weight of fresh sago frond was used for this research. The sago frond fibre was ground until it turned into fibrous form as shown in **Figure 3.4**. The fibrous sago form was then squeezed with muslin cloth to obtain the juice. Then, the juice was filtered to remove fibrous solids and scum. The filtered juice was further clarified by withdrawing the solid particles by centrifugation at 10,000 rpm for 10 minutes at 32 °C. Next, the SFJ was analysed with DNS method for initial sugar concentration.

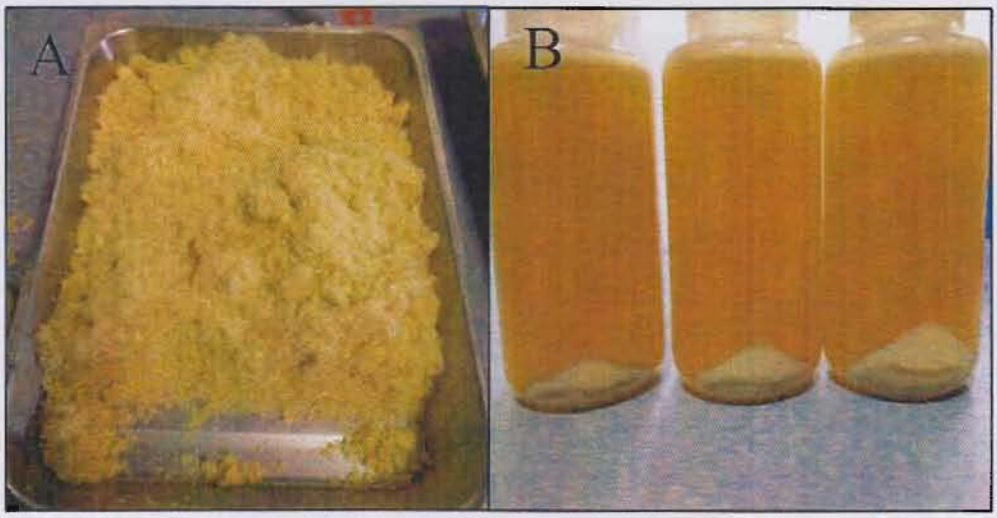

Figure 3.4 Preparation and extraction of SFJ. (A) Fibrous form sago after ground. (B) White pellet showed the starch after tested with iodine.

3.2.2 Inoculum preparation

The working medium in the conical flask consisted of 100 ml pure SFJ, and different yeast extract or nitrogen concentration; 5 and 2.5 g/L. The initial pH was adjusted to 6.0 before autoclaving.

3.2.3 *Lactococcus lactis* I0-1 preparation

Lactococcus lactis I0-1 was first propagated at 32 °C for 9 hours with agitation by transferring single colony of stock culture to 20 ml of liquid media containing 5 g/L of yeast extract and 20 g/L glucose. The pH of the liquid media was adjusted to 6.0. The culture was then centrifuged at 2500 rpm for 10 minutes. The supernatant was discarded and the cell pellet obtained was harvested as the pure *Lactococcus lactis* I0-1. **Figure 3.5** shows the *Lactococcus lactis* I0-1 transferred into universal bottles containing 5 g/L yeast extract and 20 g/L glucose.

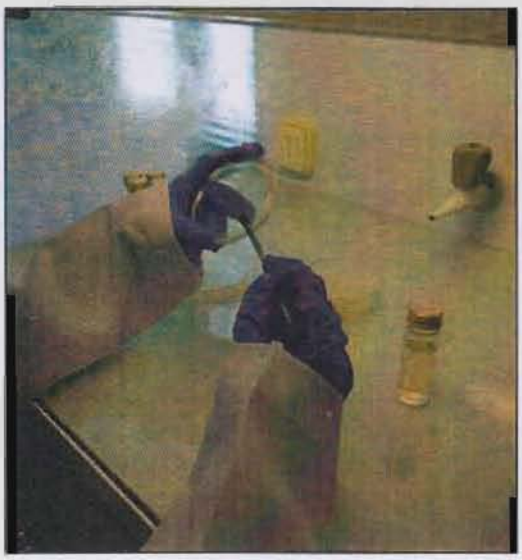

Figure 3.5 Single colony of *Lactococcus lactis* I0-1 prepared 24 hours before in potato dextrose agar was transferred into YMB of pH 6.0.