Culture of *Acetobacter aceti* TISTR 102 in Coconut Water and Banana Juice Medium for Starter Powder Preparation and Application in Coconut Vinegar Fermentation

Prince of Songkla University
Pattani Campus

Thao Ngan Ngoc Trinh

A Thesis Submitted in Fulfillment of the Requirements for the Degree of Master of Science in Food Science and Nutrition

Prince of Songkla University

2015

Copyright of Prince of Songkla University

Thesis Title	Culture of *Acetobacter aceti* TISTR 102 in Coconut Water and Banana Juice Medium for Starter Powder Preparation and Application in Coconut Vinegar Fermentation
Author	Miss Thao Ngan Ngoc Trinh
Major Program	Food Science and Nutrition

Major Advisor

…...……...

(Asst. Prof. Dr. Jaruwan Maneesri)

Co-advisor

…...…….....

(Asst. Prof. Dr. Payap Masniyom)

Examining Committee

…...……....Chairperson

(Dr. Somrak Panphon)

…...…….Committee

(Asst. Prof. Dr. Jaruwan Maneesri)

…...……..Committee

(Asst. Prof. Dr. Payap Masniyom)

…...……..Committee

(Dr. Yutana Pongpiriyadacha)

The Graduate School, Prince of Songkla University, has approved this thesis as fulfillment of the requirements for the Master of Science Degree in Food Science and Nutrition

…….……

(Assoc. Prof. Dr. Teerapol Srichana)

Dean of Graduate School

Prince of Songkla University Pattani Campus

This is to certify that the work here submitted is the result of the candidate's own investigations. Due acknowledgement has been made of any assistance received.

……..…Signature

(Asst. Prof. Dr. Jaruwan Maneesri)

Major Advisor

……Signature

(Miss Thao Ngan Ngoc Trinh)

Candidate

Prince of Songkla University
Pattani Campus

I hereby certify that this work has not already been accepted in substance for any degree, and is not being concurrently submitted in candidature for any degree.

……..…Signature

(Miss Thao Ngan Ngoc Trinh)

Candidate

Prince of Songkla University
Pattani Campus

ชื่อวิทยานิพนธ์ การเตรียมกล้าเชื้อ *Acetobacter aceti* TISTR 102 แบบผงโดยการเพาะเลี้ยงในน้ำมะพร้าวและน้ำกล้วยสกัด และการประยุกต์ใช้กล้าเชื้อหมักน้ำส้มสายชูจากน้ำมะพร้าว

ผู้เขียน Miss Thao Ngan Ngoc Trinh

สาขาวิชา วิทยาศาสตร์การอาหารและโภชนาการ

ปีการศึกษา 2557

บทคัดย่อ

การศึกษาสภาวะที่เหมาะสมในการเตรียมกล้าเชื้อ *Acetobacter aceti* TISTR 102 ในน้ำมะพร้าวผสมกับน้ำกล้วยซึ่งเป็นวัตถุดิบต้นทุนต่ำแต่ให้ปริมาณเซลล์ที่สูง โดยการศึกษาผลของอัตราการให้อากาศโดยการเขย่า ปริมาณของน้ำกล้วยสกัด และปริมาณแหล่งไนโตรเจน พบว่าน้ำมะพร้าวมีประสิทธิภาพสามารถใช้เลี้ยงเชื้อ *Acetobacter aceti* TISTR 102 เทียบได้กับการใช้อาหารเลี้ยงเชื้อ GY (glucose yeast extract broth) โดยเชื้อแบคทีเรีย *A. aceti* TISTR 102 สามารถเจริญเติบโตเมื่อเลี้ยงในน้ำมะพร้าวที่มีอัตราส่วนน้ำกล้วยผสมร้อยละ 25-100 (v/v) ($p \leq 0.05$) มีการเจริญเติบโตสูงสุดเมื่อเลี้ยงในน้ำกล้วยและน้ำมะพร้าวที่อัตราส่วนผสมร้อยละ 50 มีการเจริญเติบโตในระยะ stationary phase ในช่วงชั่วโมงที่ 18-24 มีปริมาณเซลล์ที่มีชีวิตเท่ากับ 8.82-9.07 log CFU/ml การใช้แอมโมเนียมซัลเฟตเป็นแหล่งไนโตรเจนที่ปริมาณร้อยละ 0, 0.1, 0.3 และ 0.5 ไม่ได้ส่งเสริมการเพิ่มปริมาณเซลล์ที่มีชีวิต ($p > 0.05$) ซึ่งมีอัตราการเจริญเติบโตแบบทวีคูณอยู่ในช่วง 0.196-0.204 ต่อชั่วโมง ใกล้เคียงกับการเจริญเติบโตของเชื้อแบคทีเรีย *A. aceti* TISTR 102 เมื่อใช้ยีสต์สกัดเป็นแหล่งไนโตรเจนที่ปริมาณร้อยละ 0, 0.2, 0.6 และ 1.0 ($p > 0.05$) ซึ่งมีอัตราการเจริญเติบโตแบบทวีคูณอยู่ในช่วง 0.141-0.150 ต่อชั่วโมง ในทางตรงกันข้าม การให้อากาศโดยการเขย่ามีผลต่อการเพิ่มปริมาณเซลล์ที่มีชีวิตอย่างมีนัยสำคัญทางสถิติ ($p \leq 0.05$) พบว่า เมื่ออัตราการเขย่าเพิ่มขึ้นปริมาณเซลล์ที่มีชีวิตเพิ่มขึ้น ซึ่งอัตราการเขย่าที่ความเร็ว 150 รอบต่อนาที ทำให้มีปริมาณเซลล์ที่มีชีวิตอยู่ในระยะ stationary phase เท่ากับ 8.56-8.67 log CFU/ml ในขณะที่การเขย่าที่อัตรา 120 รอบต่อนาที มีปริมาณเซลล์ที่มีชีวิตประมาณ 8.46-8.57 log CFU/ml

การศึกษาการใช้น้ำตาลซูโครสเป็นสารปกป้องเซลล์ที่ระดับความเข้มข้นร้อยละ 0, 10, 20, และ 30 (w/v) โดยการนำตะกอนเซลล์ *A. aceti* TISTR 102 ผสมกับสารละลายน้ำตาลซูโครส ผสมกับรำข้าวที่ผ่านการฆ่าเชื้อแล้ว นำไปอบที่อุณหภูมิ 35 องศาเซลเซียส เป็นเวลา 12 ชั่วโมง พบว่า เมื่อความเข้มข้นของน้ำตาลซูโครสเพิ่มขึ้นปริมาณเซลล์ที่มีชีวิตของ *A. aceti* TISTR 102 จะลดลง โดยมีปริมาณเซลล์ที่มีชีวิตเท่ากับ 7.78±0.10, 7.37±0.14, 6.86±0.23 และ 6.46±0.14

log CFU/g ตามลำดับ อย่างไรก็ตามปริมาณเซลล์ที่มีชีวิตหลังจากการอบแห้ง พบว่า ที่ความเข้มข้นของน้ำตาลซูโครสร้อยละ 1-20 จะมีปริมาณเซลล์ที่มีชีวิตแตกต่างกันอย่างมีนัยสำคัญทางสถิติ ($p \leq 0.05$) แต่ที่ปริมาณน้ำตาลซูโครสร้อยละ 30 นั้นไม่แสดงคุณสมบัติในการปกป้องเซลล์ ซึ่งคำนวณเป็นอัตราการรอดชีวิตเท่ากับร้อยละ 68.24±1.42, 71.24±2.04, 79.07±3.35 และ 81.50±1.91 ตามลำดับ การศึกษาผลของการใช้ซิลิกาเจลร่วมกับการเก็บรักษากล้าเชื้อ *A. aceti* TISTR 102 แบบผงโดยใส่ในซองอลูมิเนียมฟอยล์ ปิดผนึกแบบสุญญากาศ เก็บในตู้เย็น (อุณหภูมิ 4 องศาเซียลเซียส) เป็นระยะเวลา 2 เดือน พบว่า กล้าเชื้อ *A. aceti* TISTR 102 แบบผงมีปริมาณเซลล์ที่มีชีวิตเริ่มต้นเท่ากับ 5.89±0.05 log CFU/g มีปริมาณน้ำอิสระ (a_w) เท่ากับ 0.5710±0.006 และหลังจากเก็บรักษาเป็นระยะเวลา 50 วัน พบว่า มีปริมาณเซลล์ที่มีชีวิตลดลงเหลือเท่ากับ 5.65±0.05 log CFU/g มีปริมาณน้ำอิสระ (a_w) เพิ่มขึ้นเท่ากับ 0.5782±0.003 ในขณะที่ปริมาณความชื้นเริ่มต้นมีค่าเท่ากับร้อยละ 7.85±0.32 และเพิ่มขึ้นหลังระยะเวลาการเก็บรักษาวันที่ 20 โดยมีค่าเท่ากับร้อยละ 8.13±0.31

กระบวนการการหมักน้ำส้มสายชูจากน้ำมะพร้าว มี 2 ขั้นตอน ขั้นตอนแรก การหมักแอลกอฮอล์ด้วยเบเกอร์ยีสต์ และการหมักกรดอะซิติกด้วยกล้าเชื้อ *A. aceti* TISTR 102 แบบผง ในกระบวนการหมักแอลกอฮอล์ พบว่า ความเข้มข้นของปริมาณน้ำตาลเริ่มต้น และปริมาณกล้าเชื้อยีสต์มีผลต่อประสิทธิภาพการหมัก โดยการเพิ่มปริมาณกล้าเชื้อยีสต์ส่งผลให้ประสิทธิภาพการหมักเพิ่มขึ้น และระยะเวลาการหมักลดลง โดยการใช้ปริมาณกล้าเชื้อยีสต์ร้อยละ 0.2, 0.4 และ 0.6 (w/v) เมื่อสิ้นสุดระยะเวลาการหมัก พบว่า มีปริมาณเอทานอลเท่ากับร้อยละ 9.92±0.18, 9.81±0.18 และ 9.86±0.10 ภายในระยะเวลาการหมัก 3, 2 และ 2 วัน ตามลำดับ ในทางกลับกัน การเพิ่มปริมาณน้ำตาลเริ่มต้นในการหมักส่งผลให้ปริมาณเอทานอลเพิ่มขึ้น และระยะเวลาในการหมักเพิ่มขึ้น การหมักเอทานอลในน้ำมะพร้าวที่มีปริมาณน้ำตาลเข้มข้น ร้อยละ 12, 16 และ 20 (w/v) เมื่อสิ้นสุดระยะเวลาการหมัก พบว่า มีปริมาณเอทานอลร้อยละ 6.27±0.14 (v/v), 9.61±0.17 (v/v) และ 12.37±0.06 (v/v) ภายในระยะเวลา 1, 2 และ มากกว่า 3 วัน ตามลำดับ ดังนั้นสภาวะที่เหมาะสมในการหมักแอลกอฮอล์ในน้ำมะพร้าว คือ มีปริมาณน้ำตาลร้อยละ 12 (w/v) และปริมาณกล้าเชื้อยีสต์ร้อยละ 0.4 (w/v) ซึ่งสามารถหมักเอทานอลร้อยละ 6 (v/v) ภายในระยะเวลา 1 วัน โดยใช้เอทานอลเป็นสับเตรทสำหรับการหมักกรดอะซิติกด้วยเชื้อ *A. aceti* TISTR 102 แบบผง พบว่า การใช้กล้าเชื้อ *A. aceti* TISTR 102 แบบผงที่ความเข้มข้นร้อยละ 0.5 สามารถหมักให้ปริมาณกรดอะซิติกเท่ากับ 6.27±0.02 กรัม/ 100 มิลลิลิตร ภายในระยะเวลา 18 วัน คิดเป็นประสิทธิภาพการหมักเท่ากับร้อยละ 89 การทดสอบทางประสาทสัมผัสของน้ำส้มสายชูจากน้ำมะพร้าว พบว่า ผู้ทดสอบชิมให้การยอมรับทุกคุณลักษณะของน้ำส้มสายชู โดยผู้ทดสอบชิมให้คะแนนด้าน กลิ่น ความเปรี้ยว และความชอบโดยรวมสูงเมื่อเทียบกับน้ำส้มสายชูจากตาลโตนด

Prince of Songkla University Pattani Campus

Thesis Title	Culture of *Acetobacter aceti* TISTR 102 in Coconut Water and Banana Juice Medium for Starter Powder Preparation and Application in Coconut Vinegar Fermentation
Author	Miss Thao Ngan Ngoc Trinh
Major Program	Food Science and Nutrition
Academic year	2014

ABSTRACT

The culture of *Acetobacter aceti* TISTR 102 in coconut water with banana juice was studied to optimize culture conditions for the highest cells with low cost production. The effects of supplementary banana juice, ammonium sulfate, yeast extract and shaking speed were investigated. Coconut water was a potential culture medium for *A. aceti* TISTR 102 with the viable cells comparable to those in synthetic GY (glucose and yeast extract) medium. Banana juice volume from 25% to 100% (v/v) in coconut water showed the significant effect on the bacterial growth ($p \leq 0.05$). The highest cell viability attained in coconut water supplemented with 50% (v/v) banana juice: 8.82-9.07 log CFU/mL in the stationary phase within 18-24 hrs. The ammonium sulfate at concentrations of 0, 0.1, 0.3 and 0.5% (w/v) did not enhance the cell viability ($p > 0.05$) with the specific growth rates ranged from 0.196 hr^{-1} to 0.204 hr^{-1}. Similarly, the cells grew ineffectively in the presence of yeast extract concentrations of 0, 0.2, 0.6 and 1.0% ($p > 0.05$); the specific growth rates ranged from 0.145 hr^{-1} to 0.150 hr^{-1}. In contrast, the shaking speed significantly affected to the cell viability ($p \leq 0.05$): the higher shaking speed was applied, the higher cell viability was obtained. The culture in stationary phase attained 8.56-8.67 log CFU/mL at 150 rpm; while 8.46-8.57 log CFU/mL was recored at 120 rpm.

The study on protective effect of commercial sucrose (Milk Phor) was performed in various concentrations 0, 10, 20 and 30% (w/v) sucrose. The *A. aceti* TISTR 102 pellet was resuspended into the sucrose solutions and then mixed with sterilized rice bran as the carrier for subsequent drying process at 35^{o}C in 12 hrs. The increase in sucrose concentrations from 0 to 30% (w/v) sucrose firstly reduced the viable cells in suspension, resulted in decreasing in initial viable cells of starter powder with 7.78±0.10, 7.37±0.14, 6.86±0.23 and 6.46±0.14 log CFU/g, correspondingly. However, after drying, the increase in sucrose concentrations from 0

to 20% (w/v) significantly increased the survival rate of bacteria ($p \leq 0.05$); but the higher concentration at 30% (w/v) sucrose did not show significantly protective effect anymore. Indeed, the survival rate was recorded at 68.24±1.42, 71.24±2.04, 79.07±3.35 and 81.50±1.91 (%) at 0, 10; 20 and 30% (%) sucrose, respectively. The *A. aceti* TISTR 102 starter powder in vacuum-aluminum sealed pouch with presence of silica gel could be stored up to 2 months at refrigerated temperature (4^{o}C). The cell viability of starter powder was initially 5.89±0.05 log CFU/g that statistically remained with 5.65±0.05 log CFU/g after 50 days storage. The water activity was 0.5710±0.006 and insignificantly increased to 0.5782±0.003 during storage time. The starter powder contained 7.85±0.32 (% wb) moisture content that slowly increased to 8.13±0.31 (% wb) after 20 days and statistically remained in storage period.

The coconut water was fermented into vinegar through two stages: alcoholic fermentation with baker's yeast and acetous fermentation with *A. aceti* TISTR 102 starter powder. In alcoholic fermentation, the baker's yeast concentration and the sugar content had significant effects on the fermentation performance. Concretely, the increase in baker's yeast concentrations significantly increased the fermentation rate, leading to reduction in fermentation time. The maximum ethanol content attained 9.92±0.18, 9.81±0.18 and 9.86±0.10 (%) with 0.2, 0.4 and 0.6% (w/v) baker's yeast after 3, 2 and 2 days, respectively. In the other hand, the increase in sugar content significantly increased the ethanol concentration and took longer time to complete. At 12%, 16 and 20% (w/v) sugar content, the ethanol content was 6.27±0.14 % (v/v) after 1 day, 9.61±0.17 % (v/v) after 2 days and 12.37±0.06 % (v/v) after more than 3 days, respectively. In alcoholic fermentation, coconut water at 12% (w/v) sugar content and 0.4% (w/v) baker's yeast were adequate for approximately 6% (v/v) ethanol concentration within 1 day. The ethanol then was the substrate for acetification with *A. aceti* TISTR 102 starter powder. The *A. aceti* TISTR 102 starter powder at 0.5% (w/v) completely produced acetic acid with 6.27±0.02 g/100 mL within 18 days, attaining 89% fermentation efficiency. For sensory evaluation, the sample fermented from coconut water was rated with acceptable scores for all of the sensorial attributes. Moreover, it also exhibited the higher scores for odor, sourness and overall acceptance, as compared to the sample fermented from commercial palm sap.

Prince of Songkla University Pattani Campus

ACKNOWLEDGEMENT

First of all, the biggest thanks I would like to convey to my great advisor Asst. Prof. Dr. Jaruwan Maneesri for her dedicated guidance and constructive suggestion for my research. She always encouraged me in working, took me care in the life and gave me useful critiques in keeping my progress on schedule. Moreover, being a part of her team, I also was prepared to be master about the microbiological knowledge that would be useful for my academic study as well as for my future career.

Also, I desire to extend my gratitude to Asst. Prof. Dr. Payap Masniyom, my co-advisor for his consultancy, especially in and experimental design and sensory evaluation. Whenever I got stuck, he was willing to support idea to keep my laboratory working on progression. He also dedicatedly helped me in correcting my thesis and my manuscript also. Besides, I am deeply grateful for the other committees: Dr. Somrak Panphon and Dr. Yutana Pongpiriyadacha for many comments to improve my academic knowledge and successfully complete my project.

I sincerely thank Mrs. Netnapid Ongsuwan, the Head of Department of Food Science and Nutrition, who gave me more knowledge and consultancy on chemical laboratory and sensory evaluation. Besides, she also organized a lot of activities from which I could know more about Thai culture and introduced me some amazing landmarks for travelling.

I would like to acknowledge Faculty of Science and Technology, Prince of Songkla University (PSU), Pattani Campus for their financial support granted during course of Master Degree. I also gratefully remember to Graduated School, Prince of Songkla University, Thailand and Halal Food Center for providing budget and equipment to conduct experiment. A deep and sincere gratitude is given to lecturers and technical staffs in Department of Food Science and Nutrition for providing the facilities and several academic comments for my experiments. Without their help, it will be no doubt that I cannot finish my project and graduate master degree.

The grateful acknowledgement is extended to Ms. Siriporn Atnarong and Mr. Manumaeri Liming who always stranded by me whenever I have trouble in laboratory and in healthy issues. Unforgettably, I also thank all Master students and research assistants in Department of Food Science and Nutrition: Miss Leena Ngafa, Miss Rusmya Arlee, Ms Suhainee Benchahem, Miss Deela Thoh, Miss Rohaya Maha, Mr.

Prince of Songkla University Pattani Campus

Madan Kumar Chapagai and Mr. Alyas Doloh, who together with me overcame difficulties in studying and doing laboratory.

Many thanks are also given to Mr. Vuong Hung Vo, Miss Phi Thi Nguyen and Miss Van Thi Le who, together with me, created homely environment. Besides, I never forget the amazing time for travelling with Mr. Huy Vu Tran, Mr. Thanh Nhut Nguyen, Mr. Huy Thanh To and Mr. Thanh Huynh Tran. Especially, I thank Mr. Thanh Nhut Nguyen who helped me in thesis writing and encouraged me all time.

Importantly, I would like to dedicate this thesis to my dear family. With their loves, I would have been able to motivate myself doing the best I can and the thesis also would have been completed well.

Thao Ngan Ngoc Trinh

Prince of Songkla University
Pattani Campus

TABLE OF CONTENTS

Prince of Songkla University Pattani Campus

Prince of Songkla University Pattani Campus

Prince of Songkla University Pattani Campus

LIST OF TABLES

Prince of Songkla University Pattani Campus

Prince of Songkla University Pattani Campus

LIST OF FIGURES

Prince of Songkla University Pattani Campus

Prince of Songkla University
Pattani Campus

CHAPTER 1
INTRODUCTION

1.1 Research background

In tropical and subtropical regions, concretely in South-East Asia countries, coconut (*Cocos nucifera* L.) is available in several varieties with a relatively large planting area. It has provided a lot of substantial benefits for economy and human life. Among the edible parts of coconut, coconut water is notably used in processing and increasingly consumed because of its delicious taste and essential nutrients. However, the usage of coconut water depends on the maturity or age of coconut fruit. Young and mature coconut water are suitable for ready-to drink beverage or freshly serving, whereas overly-mature coconut water normally is disposed after dehusking for production of coconut oil or coconut milk. According to Unagul *et al*. (2007), in Thailand, there is a large number of the waste from coconut water yearly with the reported data up to 200,000 tons and the number has been still increasing. No matter how good in control and how much in utilization, there are much more amount of overly-mature coconut water is wasted. Thereby, fermenting coconut water into vinegar is considered to be an attractive means of utilizing waste from coconut industry.

Generally, in fermentation, starter culture has been preferentially used for improvement in fermentation efficiency and for controlling the final products' qualities. The concept of starter culture in powder form (starter powder) has been recently available in market (e.g. baker's yeast) for conveniences in use, storage and preservation. Particularly, for vinegar fermentation, starter powder with acetic acid bacteria strain *Acetobacter aceti* TISTR 102 was investigated and produced by low-temperature thermal drying process (Wongsudaluk, 2012). A high cell biomass is a requisite issue and culture medium is considerably taken account in starter culture preparation (Ndoye *et al*., 2009). The mixture of coconut water and banana juice with ratio at 1:1 (v/v) was primarily reported as a cheap and nutritional inoculum media for cultivation of acetic acid bacteria as compare with synthetic media (Supakod and Wongwicharn, 2012). It is a novel medium with low cost for microbial mass

Prince of Songkla University Pattani Campus

production that successfully replaces the conventional media and potentially applied for large scale production of starter culture, and starter power in particular.

This research project aims to optimize the culture medium for *Acetobacter aceti* TISTR 102 from coconut water and banana juice to obtain the high cell viability for starter powder preparation. The effects of sucrose as a protectant and packaging with silica gel were subsequently investigated to improve the viable cells of starter powder in preparation and storage. Additionally, for application, the starter powder *A. aceti* TISTR 102 and baker's yeast were used to produce vinegar from coconut water. The fermentation conditions were optimized to develop a convenient process for application in household scale with the required acetic acid content about 4% (w/v).

1.2 Research objectives

This research has its general objective to culture *A. aceti* TISTR 102 in a cheap culture medium for starter powder preparation and to apply the starter powder into coconut vinegar fermentation

1. To optimize the culture conditions for *A. aceti* TISTR 102 in coconut water and banana juice medium
2. To study the effect of sucrose as a thermal protectant during drying process and effect of silica gel in storage *A. aceti* TISTR 102 starter powder at refrigerated temperature (4^{o}C)
3. To study the potentiality for using baker's yeast and *A. aceti* TISTR 102 starter powder in coconut vinegar production

Prince of Songkla University Pattani Campus

CHAPTER 2
LITERATURE REVIEW

2.1 Coconut (*Cocos nucifera* L.)

The coconut, botanically known as *Cocos nucifera* L., extensively grows in over the world and especially considered as an ubiquitous sight in all tropical and subtropical regions with 93% of the total coconut cultivation area in the world (Punchihewa and Arancon, 2006). It is one of the most important fruit tree, and even called "the tree of life" because of its substantial applications in human living. Coconut fruit is composed from 4 parts, including exocarp, mesocarp, endocarp and endosperm, considerably that contribute into the diversification of coconut products. The first three parts mostly used to make many industrial and commercial products or to indicate for the maturity of the fruit; while the most inner part-endosperm plays an important role in food and beverage industry. For endosperm, a fleshy part attaching into inner endocarp called coconut meat which mostly used to produce coconut oil, coconut milk, etc. Otherwise, coconut water, a clear and sweet liquid, filling up the interior hollow space, is favor to serve directly or to process into drinking products.

In particular, coconut water is reported to be a nutritious solution with sugars, proteins, antioxidants, vitamins and minerals, which are necessary for human body (Victor, 2013). Depending on maturity of coconut fruit, compositions in coconut water are varied differently. Prades *et al*. (2012) found that coconut fruit began to form a thin layer of jelly around the inside of endocarp at 5-month age and fully maturity attained after 12 months. After this time, coconut fruit was overly mature with deterioration in nutrients and volumetric quantity. Table 1 shows the compositions in coconut water at different maturity stages. As compared to immature coconut (IMC) and mature coconut (MC), overly-mature coconut (OMC) has much more differences in its component quantification. It contains less water, which was explained by thickening of kernel for replacement of water when interior pressure is released during the ripening (Prades *et al*., 2012). Jackson *et al.* (2004) pointed out the more turbidity value in OMC related to the increment of total solids concentration that associated with concentration of ions such as phosphate, sulfate, chloride, etc. In addition, they also described the taste of OMC water was slightly saltier than the taste

Prince of Songkla University Pattani Campus

of the counterparts. However, according to Shivakumar and Vijayendra (2006), it was considered as a potential microbial substrate because of the high concentrations of sugar, trace elements and nitrogen.

Table 1 Composition and physicochemical properties of coconut water
(IMC: immature coconut; MC: mature coconut and OMC: overly-mature coconut)

Physicochemical properties	Coconut maturity stage		
	IMC	MC	OMC
Volume of water (mL)	684 ± 27.0[a]	518 ± 14.2[b]	332 ± 19.9[c]
TSS (°Brix)	5.60 ± 0.14[b]	6.15 ± 0.21[a]	4.85 ± 0.17[c]
TA[d] (%)	0.089 ± 0.004[a]	0.076 ± 0.008[b]	0.061 ± 0.003[c]
pH	4.78 ± 0.13[c]	5.34 ± 0.12[b]	5.71 ± 0.10[a]
Turbidity	0.031 ± 0.013[c]	0.337 ± 0.108[b]	4.051 ± 0.323[a]
Sugar content			
Fructose (mg/mL)	39.04 ± 0.824[a]	32.52 ± 0.227[b]	21.48 ± 0.21[c]
Glucose (mg/mL)	35.43 ± 0.510[a]	29.96 ± 0.243[b]	19.06 ± 0.19[c]
Sucrose (mg/mL)	0.85 ± 0.010[c]	6.36 ± 0.06[b]	14.37 ± 0.25[a]
Minerals			
Potassium (mg/100 mL)	220.94 ± 0.320[c]	274.32 ± 0.139[b]	35.11 ± 0.133[a]
Sodium (mg/100 mL)	7.61 ± 0.041[b]	5.60 ± 0.016[b]	36.51 ± 0.020[a]
Magnesium (mg/100 mL)	22.03 ± 0.069[b]	20.87 ± 0.023[b]	31.65 ± 0.038[a]
Calcium (mg/100 mL)	8.75 ± 0.045[c]	15.19 ± 0.028[b]	23.98 ± 0.054[a]
Iron (mg/L)	0.294 ± 0.082[b]	0.308 ± 0.011[b]	0.322 ± 0.049[a]
Protein (mg/mL)	0.041 ± 0.007[b]	0.042 ± 0.002[b]	0.217 ± 0.018[a]
TPC[e] (mg/L)	54.00 ± 3.135[a]	42.59 ± 0.834[b]	25.70 ± 1.756[c]

Comparison within the rows was shown in the table with the data written as mean ± standard deviation ($n = 3$). Means followed by the same superscript letter are not statistically significant at the 5% level.
[d] Titratable acidity expressed as malic acid percentage.
[e] Total phenolics content expressed as mg GAE/L.

Source: Tan *et al.* (2013)

Unagul *et al*. (2007) added coconut water into standard medium with various ratios to observe its effect on biomass and docosahexaenoic acid (DHA) produced by *Schizochytrium mangrovei* Sk-02. The biomass level and product formation with 28 g/L and 6 g/L, respectively were obtained in the medium with 33% (v/v) added coconut water. These results were higher, even up to 50% as compared with those from the non-supplemented culture medium. In addition, there was a synergistic effect on improvement of biomass production between nitrogen sources (casitone, peptone or tryptone) and 50% (v/v) added coconut water. Otherwise, the poor growth was observed with the same nitrogen sources in media without coconut water addition.

Seesuriyachan *et al*. (2011) used coconut water as a component of modified-MRS medium for reduction the cost in cultivation of *Lactobacillus confusus* TISTR 1498. In the presence of coconut water, with only 20 g/L sucrose addition, the

biomass was 50% higher than MRS medium supplemented with 100 g/L sucrose. Moreover, the highest yield of exopolysaccharide (EPS) and high cell biomass were obtained in coconut water supplemented 20 g/L sucrose, while the quantity of three expensive supplements (peptone, yeast extract and beef extract) was reduced to 50% (w/v).

Supakod and Wongwicharn (2012) proved the good growth patterns of acetic acid bacteria in natural media as compared with conventionally synthetic media (HS and GEY). Coconut water (a by-product of coconut industry) and banana juice hydrolyzed by enzymes individually archived at least 10^6 CFU/mL after 6-12 hours that comparable to those archived in synthetic media. The mixture of coconut water and banana juice with ratio 1:1 was even reported to perform the highest growth patterns. An example for growth patterns of *Acetobacter aceti* TISTR 102 in different media is shown in Figure 1. In respect to economy, the price for 1L medium of coconut water, banana juice and mixture of them were 1 baht, 11.48 baht and 6.74 baht, respectively. Otherwise, one liter of synthetic media costs 14.90 baht for GYE medium and 42.22 baht for HS medium. Therefore, agricultural substrates such as coconut water and banana juice potentially produce starter culture with comparative cost and high efficacy.

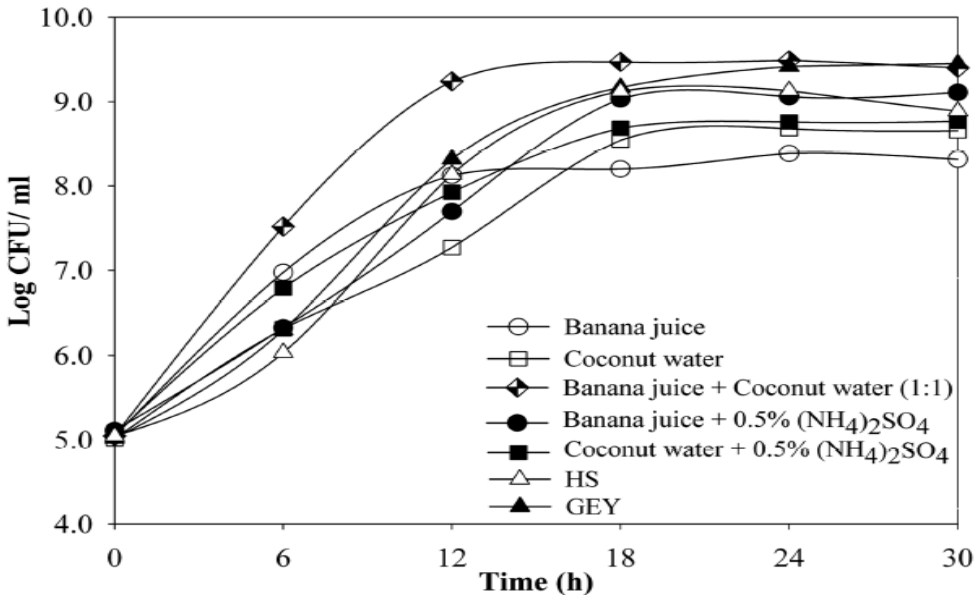

Figure 1 Growth patterns of *Acetobacter aceti* TISTR 102 in different culture media
Source: Supakod and Wongwicharn (2012)

2.2 Banana (*Musa sapientum*)

Bananas are popular edible fruits with a seasonal crop and mostly distributed in tropical regions. The poor post-harvest handling, the rapid rate of deterioration and long transportation are notable issues in banana storage, leading to wasting a large quantity of bananas. Extracting bananas with hot water at the ratio 1 banana pulp:1 water at 95°C to produce juice also has been an effective utilization (Lee *et al*., 2006). The fully ripe bananas with a high carbohydrate content is a potential carbon source for food fermentation such as banana wine (Onwuka and Awam, 2001; Akubor *et al*., 2003) or banana vinegar (Loesecke, 1929) with high score from consumer acceptance test. In terms of microbiology, banana has been also a cheap and efficient substrate for biotechnological production process. For examples, *Aspergillus niger* ORS-4 produced 39.6 g/L gluconic acid with 40% yield and 0.895g/L/d in banana must that significantly higher than data obtained in untreated molasses and starch (Singh *et al*., 2005).

Lyte (1997) stated that banana contained large quantities of neurochemicals that possibly improve the bacterial growth. The effects of banana's extracts (*Musa x paradisiaca*) (e.g from peel and pulp at different stages of ripening: unripe, ripe and overripe) on several bacteria, including non-pathogenic (e.g *E. coli* ML-1 and *E. coli* MB-1) and pathogenic bacteria (e.g. *E. coli* O157: H7; *Shigella flexneri* MD 12022, *Enterobacter cloacae* ATCC 23355, *Salmonella typhirium* ATCC 14028) were investigated. Representative, *S. flexneri* and *E. coli* ML-1 cultures in 0.1% (v/v) overripe pulp and peel extract increased their growth within 24 hours as compared to the cultures in 1.0% (v/v) extraction medium (vehicle). He explained that the presence of norepinephrine and dopamine in banana extract possibly modulated the bacterial growth. Indeed, the *S. flexneri* culture with exogenous norepinephrine and dopamine showed the higher cell viability with 3 log-fold increases as compared to the cultures in non-supplemented media. Also, norepinephrine was available in unripe, ripe and overripe banana pulp extract with 295, 825 and 325 ng/ml extract, respectively; while dopamine data was not shown. Total organics was recorded correspondingly 38.1; 50.7 and 43.4 mg/ml extract.

Prince of Songkla University Pattani Campus

Freestone *et al*. (2009) researched the enhancement effects of banana extract, especially from pulp extract on bacteria (i.e. lactic acid bacteria - LAB). They pointed out that banana extract was resistant to heat treatment, even at autoclaved condition (121°C for 20 minutes) without significant loss of activity. Experimentally, the *L. casei* strain was cultured in serum-based medium with supplemented banana extract and with individually dietary phenolates (e.g tannic acid, catechin. caffeic acid, chorogenic acid) which able to modulate the LAB growth. The banana extract obviously showed a comparably microbial growth; and the others with purified form were expensive and less effective. Moreover, they pointed out that lactic acid bacteria took at least 5 days to grow with individual colony size on Luria Agar (LA); while 2% (v/v) banana extract supplemented in LA agar, the incubation time could be remarkably reduced to only single overnight at 37°C. Also, at all serially–diluted levels, their visible colonies on MRS agar with 2% banana extract were substantially more than colonies on non-supplemented MRS agar after 36 hours under anaerobic conditions at 30°C. Furthermore, banana extract even improved cell viability in food (e.g yogurt) with approximately higher over 140-fold viable cells obtained. Indeed, there was a significant difference about level of viable cells occurring in presence of banana extract ($p<0.001$): the significant increase appeared after 3 days and peaked at about 7 days. In contrast, the non-supplemented yogurt did not show the significant changes in viable cells during incubation time.

Additionally, as previously mentioned, enzyme-extracted banana juice was used to culture some acetic acid bacteria strains (Supakod and Wongwicharn, 2011). Banana juice showed comparably growth patterns as compared to those in some synthetic media (HS and GYE) or coconut for most of bacterial strains, exception for *A. xylinum* Coc5. The presence of ammonium sulfate as supplemented nitrogen source at 0.5% (w/v) affected positively (*A. xylinum* Coc5) and negatively on the cell viability (*A. xylinum* TISTR 975 and *A. xylinum* AGR 60), depending on the bacterial strains. Although there was no special or detailed explanation for effects of banana juice, its promoting effects on biomass was confirmed.

Prince of Songkla University Pattani Campus

2.3 Starter culture dehydration

2.3.1 Starter culture

A large number of variable microorganisms such as bacteria, yeasts, molds, ect added into the substrate to accelerate the fermentation process and/or to facilitate the predictability of its products is defined as starter culture (Holzapfel, 1997). To obtain an efficient starter, microorganisms are isolated from specific substrate (e.g foods) to screen their characteristics. The desirably purified strains are identified and kept for maintenance. The pure culture usually proliferates in small-volume medium to attain approximately 10^6 cells/mL or more for sufficiently being starter culture for fermentation (Ray, 2004). Starter culture includes one microorganism (single-strain culture) or even mixture of different strain cultures (multiple-strain culture). Some examples are showed in Table 2.

Table 2 Types of starter cultures used in various fields of food fermentation typical of industrialized countries

Foodstuff	Single-strain culture	Multiple-strain culture	Mix-strain culture
Sauerkraut	+	-	-
Various vegetable	+	-	-
Vegetable juice	+	-	-
Soy products	+	-	-
Sour dough	+	+	+
Wine	+	+	-
Dry sausage	+	+	-
Dairy product	+	+	+

(+), applied and (-), not used

Sources: Buckenhuskes (1993 cited by Holzapfel, 2002)

As mentioned above, acceleration in fermentation process and ability to control product quality are the primary considerations for starter selection. Besides, Holzapfel (2002) also mentioned to further features for starter culture, including non-toxinogenicity and non-pathogenicity, stability and maintenance its activity even after prolonged subculturing, anti-activity to pathogens, toxic, residues, etc and probiotic properties.

Prince of Songkla University Pattani Campus

2.3.2 Starter culture dehydration

Drying is defined as the process in which the energy given by hot air is received by sample, and the moisture in sample is taken by dry air at the same time (Heldman and Hartel, 1997). In recent years, drying has been widely applied for microorganisms with maintenance their biological activity for increasing of trading values. Moreover, dehydrated starter culture has been preferred in use due to advantages in application and storage.

According to Fu and Chen (2011), during a thermal drying process, most of microorganisms are affected by two significant stresses including heat and dehydration. With heat, macromolecules such as protein, amino acids, etc are denatured in manner of breaking their internal linkages into monomeric units which are easier destructed in further, results in the death of cells. Otherwise, dehydration stress not only removes the water available in cytoplasmic, leading to the change of physical state; but also causes lipid peroxidation for leaking some of life-essential cellular substances. They summarized some extrinsic factors could affect to the cell survival during dehydration, even in storage time that were presented in Figure 2.

The increase in survival cells has been important strategy to improve the quality of dehydrated starter culture. The main concepts are to protect the essential cellular structure intact from the causative damages in drying and to keep the fully functional activities after rehydration to maintain the cell viability. In fact, the heat stress and thermal injury are unavoidable; each applied drying process has specific parameters need to be controlled for enhancement survival rate. For examples, *Lactobacillus paracasei* cultured at a pilot scale (300 L) could survive up to 84.5% within 80 to 85°C optimal air outlet temperature of for spray drying. They were present in powder with moisture contents from 4.1 to 4.2% and viable counts of 3.2×10^{9} CFU/g (Gardiner *et al*., 2002). Friesen *et al*. (2005) pointed out convective air-drying for *Penicillium bilaiae* - a fungal micro-organism, was affected by solids temperature, moisture content and drying rate. The viable conidia were significantly recovered with the high inlet air relative humidity and slower drying rate. However, in this part, the drying processes and controlling their parameters are not referred to; while the general concepts that could be concerned before and after drying to protect/enhance the microbial cells were especially mentioned.

Prince of Songkla University Pattani Campus

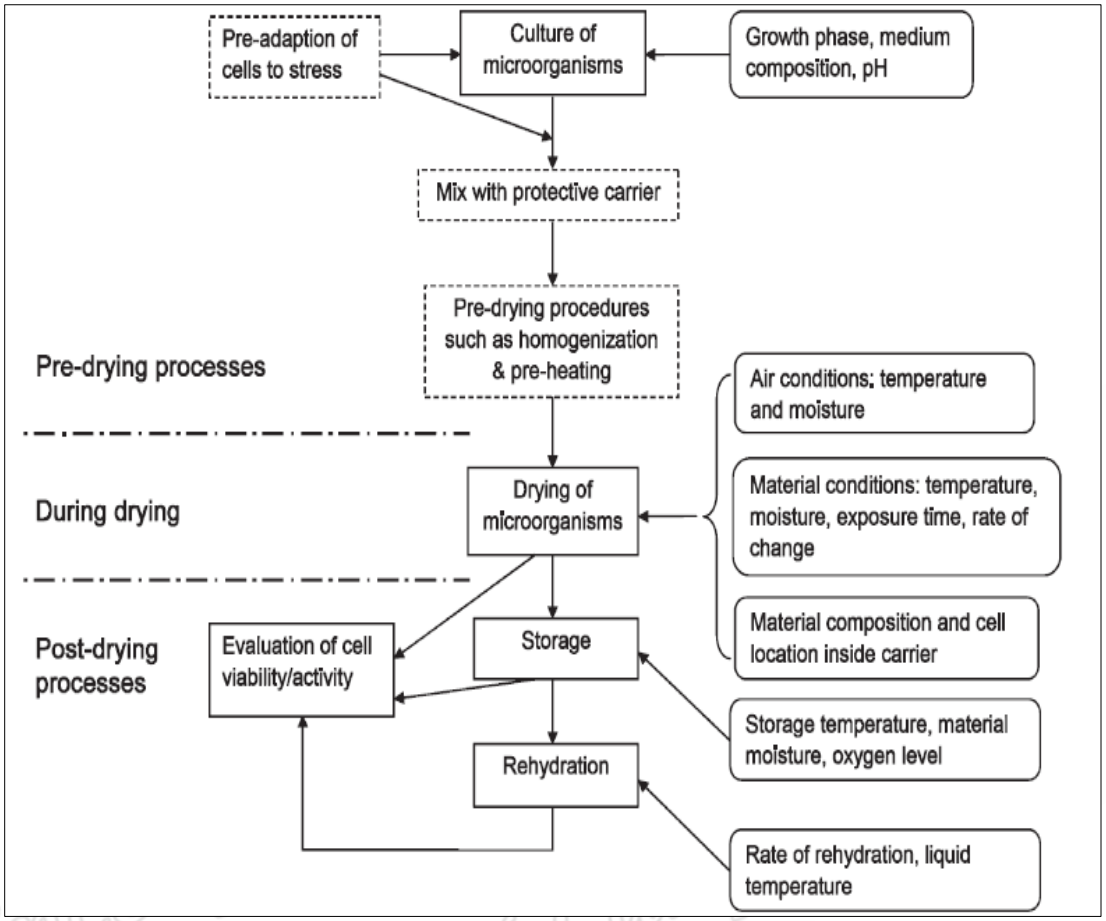

Figure 2 Important extrinsic factors in each processing step during the dehydration of microorganisms
Source: Fu and Chen (2011)

Corcoran *et al.* (2004) reported that *Lactobacillus rhamnosus* GG at stationary phase could exhibit better survival than at other phases for the spray-drying process; bacteria at the lag phase were the most susceptible to environmental effects. The explanation possibly was due to the high stress tolerance induced by physiological changes of growth medium such as carbon starvation, deprivation of nutrients and accumulation of toxic metabolites of this phase. Besides, culture medium with induced stressful shock responses (e.g. osmotic shock, heat shock) temporary improved the stress tolerance that might improve cell viability during subsequent drying and storage. Tymczyszyn *et al.* (2007) studied the growth of *L. bulgaricus* in low water activity medium (e.g. MRS broth with 250 mmol/L sucrose) with trehalose and sucrose as protective agents during drying process. They found the synergic

protective effects on microbial survivals as they primarily were cultured in osmotic-shock medium and consequently suspended into protective agents.

Protectant is the term indicates for the substance added to protect microorganisms/cells from thermal intact during drying. They have been classified into several types such as monosaccharide, disaccharides and polysaccharides, proteins, peptides, etc which are applied specifically for microorganisms. In addition, they physically are present in liquid and solid forms, while the uses largely depend on drying process. Fu and Chen (2011) reported the cells suspended into liquid protectants were protected from thermal intact by stabilizing the cellular structure by accumulation of compatible solutes or osmolytes (e.g. glutamate, proline, betaine, carnitine, sucrose, trehalose) to alleviate the heat and osmotic stress. Otherwise, solid protectants reduced water activity of the pellet could cause the osmotic shock for the cells prior to drying. The temporary shock might improve the stress tolerance that might improve cell viability during subsequent drying. Their protective effects were showed in several studies in which the survival cells suspended in protectant is higher than the cells in distilled water (Nodye *et al*., 2007, Wongsudaluk, 2012).

Storage conditions including temperature and packaging that closely related to moisture content, water activity and oxygen significantly affected on viable cells during storage. Four degree Celsius has been reported to be the recommended storage temperature for many microorganisms. For examples, freeze-dried cells of thermal-resistant strains of acetic acid bacteria could be conserved for at least 6 month without loss of viability (Nodye *et al*., 2007). *Acetobacter aceti* TISTR 102 starter powder prepared by low-thermal drying method could remain their viability within 1 month at refrigerated temperature (Wongsudaluck, 2012). Otherwise, at elevated temperature such as 37^{o}C caused significant reduction in cell availability, which properly explained by the natural degradation of life-essential macromolecules such as lipids and proteins. In contrast, moisture content and oxygen presence favorably proceed to biochemical reactions that cause the decline in viable cells during storage. Indeed, moisture content is reported to cause decrease viable cells at the high levels (Teixeira *et al*., 1995; Ying *et al*., 2010). The vacuum condition could remain the cell viability better than the without-vacuum condition could (Chávez and Ledeboer, 2007, Wongsudaluk, 2012).

2.4 Vinegar

2.4.1 History and market trends

The word "vinegar" originates from the old French which means “vinaigre” or “sour wine”. Historically, vinegar firstly appeared thousand years ago when wine accidentally had gone bad with over time. It is possibly considered as a food by-product from wine (alcoholic substrate) when wine is further fermented in contact with air by bacterial organisms (Chatterjee, 2009). Vinegar is present globally with various types in market depending on culture and region. The dressing and vinegar industry continuously develops with more and more new product launches have been introduced in over the world. The new product launches in 2008 are presented in Table 3 with some interesting features corresponding to regional culture and healthy concerns. In spite of only 23% of total launches, Asia Pacific was the second in total launches as compared to the other regions in 2008. Thailand, Vietnam and China were reported as being the most active countries, together accounting for 56% of new product launches in Asia. From this result, in Asia countries, these three countries in particular, have opportunities to introduce their products into global market. However, they also face to challenges to make products that possess the proposed features and distinctive flavor.

2.4.2 Uses of vinegar

Vinegar, the oldest cooking ingredient in the world, is superior for dissolving essential oils from herbs, spices to make a sauce ingredient with its ubiquitous uses in culinary (Conner and Allgeier, 1976). Today, the use of vinegar has been still applied not only in household scale (e.g. marinades), but also in dressing industry with enormous products such as salad dressing, mayonnaise, ketchup, hot sauce, etc. In general, the uses of vinegar are applied in food industry as an acidulant, a flavoring agent (Hutkins, 2008). In addition, due to its acetic acid content, vinegar is a preservative itself and throughout history it has been a traditionally preservative method for food, along with solar drying, salting and smoking (Adam, 1998). By adjusting pH of certain food products, harmful microorganisms could be inhibited in pickling method. Until now, using vinegar for preservative purpose has been remained, especially in developed countries because of the lower cost.

Prince of Songkla University Pattani Campus

Table 3 Data of new product launches of dressing and vinegar industry in different regions

Areas	Percentage of new product launches	Features of production
Europe	37%	Producing specific vinegar varieties from Organic, blueberry, cranberry, Saskatoon berry, ice wine, maple syrup
North America	-	Focusing dominantly on naturalness
Latin America	18%	Reducing allergen and calories
Asia Pacific	23%	Reducing the fat content and the amount of additives and preservatives; naturalness and health trend
Middle East and Africa	-	Producing vinegar from specific fruit varieties apple, cranberry, raspberry, honey, and potato.

Source: http://www.ats-sea.agr.gc.ca/inter/4344-eng.htm

Since a long time ago, the use of vinegar as medicine probably started soon after it was discovered. Indeed, Babylonians and Greek used vinegar as a disinfectant for wounds or combined with honey for patients to recover from illness (Conner and Allgeier, 1976). Vinegar until now has been used for remedy purposes, however with insignificantly applied cases. In contrast, vinegar, itself is a subject of commercial product for serving as drinking beverage and using vinegar as a healthy drink has been a new trend in medicinal consumption of vinegar. The concept of drinking-vinegar appeared a long time ago in Roman when antibiotic activity predominantly emerged and it was proved to be an intoxicating drinking. The healthy benefits so far have been reported by Kim *et al.* (2012) such as immune modulation effect, suppression of cardiovascular disease, prevention of appetite increase, an increase in digestion absorption, and reduction in serum cholesterol level, arterial stiffening, and blood pressure. In terms of product development, several types of vinegar that flavored with flowers, fruits or honey are created to satisfy the drinking-vinegar market.

Prince of Songkla University Pattani Campus

Also according to Conner and Allgeier (1976), vinegar contains acetic acid as the main chemical composition is not only used in food but also applied in living. Indeed, vinegar used to apply in fire-setting system as a coolant, but now some artificial chemicals have been replaced. Several tips in which vinegar is considered as a natural beautifier for body, skin or hair are shared on network or in book likewise. White leads that are widely used to produce paints and cosmetics could be made from vinegar. Moreover, vinegar also has many healing functions such as cleaning, sanitizing and deodorizing.

2.4.3 Vinegar fermentation

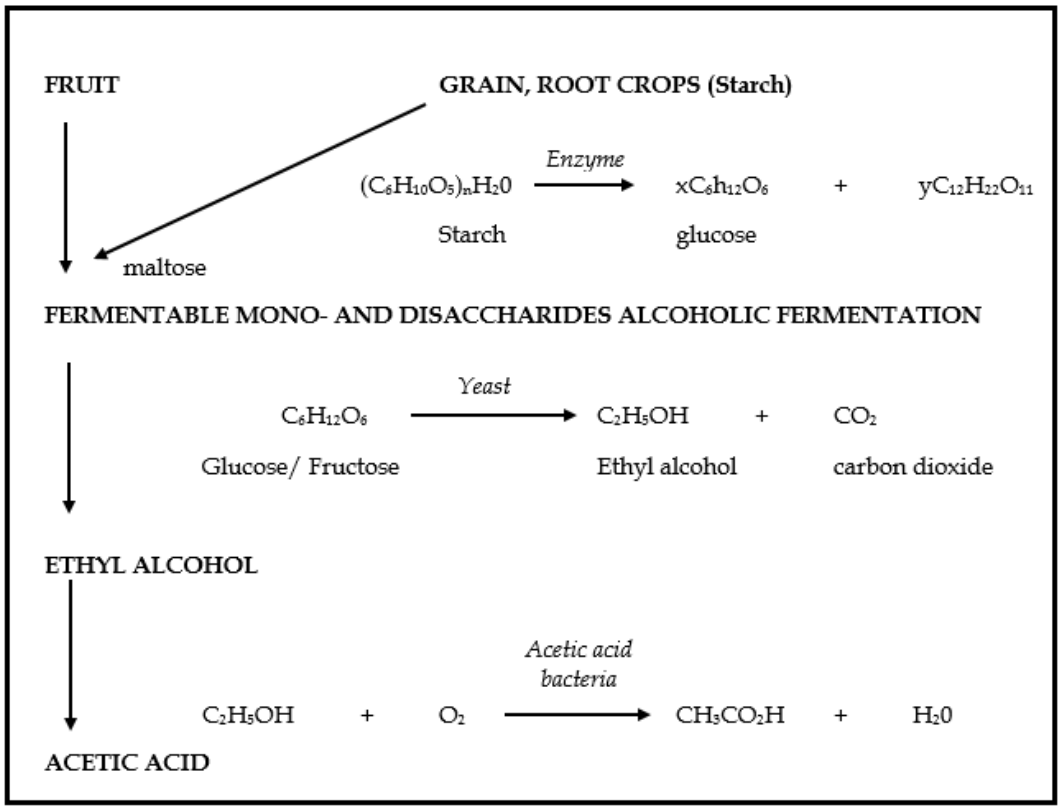

Figure 3 Schematic outline of vinegar production
Source: Adam (1998)

Figure 3 shows a schematic outline for production of vinegar-an acidic liquid made from fermentable carbohydrate source within 2 steps: ethanol fermentation and acetification. While the first stage involves activities of yeasts to convert fermentable sugar into ethanol, acetic acid bacteria oxidize ethanol into acetic acid in the second stage. With any natural sources of sugar, vinegar could be produced into several kinds

of vinegar. For example, the sugar may be derived from the juice, or cider, of fruit (such as grapes, apples, raisins, or even coconuts); from a grain (such as barley or rice); from honey, molasses, or sugar cane; or even, in the case of certain distilled vinegars, from the cellulose in wood (such as beech). Theoretically, 1g from glucose could be converted into 0.51g and 0.67g of ethanol and acetic acid, respectively. From that, 2% (w/v) is the minimum sugar content required to attain every 1% (w/v) acetic acid in the final product. Therefore, depending on the characteristic of raw materials, dilution or concentration by adding more sugar could be applied (Adam, 1998).

2.4.4 Vinegar production methods

2.4.4.1 Orleans process

This process was accidently found in 1670 when the wine spontaneously acidified into acetic acid within the long storage time under activity of wild microorganisms. In this process, the wood barrels are filled with alcohol fermenting liquid; the acetic acid bacteria developed as a slime layer on the top of the liquid. When the layer is easy to be broken when vinegar is shaken or taken out and falls down to the bottom. But, fermentation continuously occurs and the new layer will be formed. The low of acidity and very long fermentation time are disadvantages for this method. Moreover, risk from undesirable microorganism could cause spoilage for product. However, this method had an advantage in setting up with simple apparatus and high pure quality, especially for spirit (pure wine) vinegar.

2.4.4.2 Generator method

This fermenting barrel is supplemented with a non-compacting material (e.g. beech wood shavings, charcoal or coke) containing acetic acid bacteria. The fermenting liquid trickles over the packing material that is re-circulated toward the bottom while air moves from the bottom through inlets toward the top. As the result, the interact surface between the fermenting liquid, air and bacteria increases to improve the efficiency and productivity. The higher acetic acid concentration could be obtained within the relatively simple equipment and short time for fermentation. However, the packing materials should have some properties such as nontoxic, low density, high porosity, rough and large surface, and high mechanics.

Prince of Songkla University Pattani Campus

2.4.4.3 Submerged fermentation

In this method, the fermentor is designed to how the mash is stirred and aerated frequently. A heat exchanger is also set up to regularly maintain the optimum temperature during the fermentation process. In the fermentor, a suspension is formed with bacterial packing is solid phase and fermenting substrate is liquid phase. Within this design, the fermentation time is shortened to get a high efficiency. The disadvantage of this equipment is the complicated apparatus with the high cost for setting up.

2.4.5 Vinegar qualities

Hutkins (2008) found that there were two considerable criteria for assessment the quality of vinegar: physiochemical and sensorial criterion. The former associated to the properties that can differentiate vinegar from diluted vinegar or non-brewed condiments, while the later related to sensorial attributes for marketable potential.

2.4.5.1 Physiochemical properties

For vinegar, acetic acid content is the principle quality criterion that producers and consumers concern about. Titration with standard sodium hydroxide and phenolphthalein as indicator is used to determine total acetic acid content. The higher its level of acetic acid, the more acidic the vinegar will be. In UAS, acidity is set up at 4% (w/v) for minimum level, and this level is also recommended for vinegars in UK. Similarly, 4% (w/v) acidity is also applied in Asian regions, and Thailand is an example. Otherwise, in Europe region, the Codex Standard proposes 6% for wine vinegar and 5% for other types. In other words, depending on culture and region, on type of vinegar, the minimum level for acidity is different, but normally acceptable content of between 4%-5% (w/v). Vinegar is made from ethanol substrate but the maximum residual ethanol in vinegar is only 0.5% (v/v), exception wine vinegar with 1.5% (v/v). Examples of acidity and residual ethanol in some types of vinegar are shown on Table 4. Several methods based on physiochemical properties of vinegar could be used to differentiate it with distilled vinegar and non-brewed condiment. For example, pH refers to the amount of hydrogen ions present in solution is the simplest method. Vinegar with non-volatile buffering has the higher pH value as compared with observed values of equivalent acetic acid solutions. The UV absorption spectrum, osmotic pressure, freezing point depression, even some

Prince of Songkla University Pattani Campus

biochemical assessments (e.g: oxidation value, alkaline oxidation value, etc) also give rapid and certain differentiation between vinegars and other samples. Chatterjee (2009) claimed that vinegar containing 5% (w/v) acetic acid had pH, density, freezing point and boiling point of 2.4, 1.01 (g/ml), -2^{o}C (28F) and 100.6^{o}C (213F), respectively. Vinegar is composed of 95% water. It contains no protein, fat or vitamins, very little carbohydrate and very few calories. Unpasteurized vinegar contains very small quantities of minerals. Pasteurized vinegar has almost no minerals.

Table 4 Acidity and residual ethanol content of several vinegars

Vinegar	Acidity (% w/v)	Ethanol (% v/v)	References
Malt vinegar	4.3 - 5.9	-	Solieri and Giudici (2009)
Cider vinegar	3.9 - 9.0	0.03	
Wine vinegar	4.4 - 7.4	0.05 -0.3	
Rice vinegar	4.2 - 4.5	0.68	
Chinese rice vinegar	6.8 - 10.9	-	
Cashew vinegar	4.62	0.13	
Coconut water vinegar	8.28	0.42	
Mango vinegar	4.92	0.35	
Sherry vinegar	7.0	0.35	
Pineapple vinegar	5.34	-	
Jujube vinegar	3.91	2.07	Vithlani and Patel (2010)
Onion vinegar	29.4	2.00	Horiuchi *et al.* (1999)
Sugar cane vinegar	5.05	2.20	Kocher *et al*. (2014)

Prince of Songkla University Pattani Campus

2.4.5.2 Sensory evaluation

According to Gerbi *et al*. (1997), "Sensory analysis is an indispensable pre-requisite to chemical analysis, in the definition of the characteristics and value of food products". For vinegar products, several sensorial criteria relates to visual appearance (e.g. turbidity, colour), odor (smell), organoleptic attributes (e.g: flavor, sourness). However, these attributes are not easy to be assessed because of the aggressive taste and smell that specifically characterize for vinegar products.

Therefore, there have been two common manners to serve sample for sensory evaluation. The first is to serve the samples in normal tasting glasses and the taste is evaluated using a glass rod or stainless steel teaspoon to limit the quantity of vinegar ingested. On the other hand, samples are possibly diluted or mixed with other foods are usually preferential ways for carrying out. Besides, because of speciality in assessment, trained panelists and specific descriptors are required to obtain the reliable and detailed results.

2.5 Alcoholic fermentation

2.5.1 Yeast

Yeast belongs to eukaryotic microorganisms, especially the kingdom of fungi with the asexual reproduction by budding or division (Walker, 1998). To be fact that, generally there is a large number of yeast and yeast-like fungi widely distributed in nature (e.g. orchards and vineyards, in fruit and vegetable, in the air, the soil and the intestinal tract of animals) (Solieri and Giucidi, 2008), but only a few are commonly associated with the production of fermented foods. The genus *Saccharomyces* was important in food fermentations due to its varieties were the most common yeasts in fermented foods and beverages based on fruit and vegetables (Walker, 1998). Moreover, even many of the fundamentals of genetic inheritance in eukaryotic cells were initially identified and studied in this yeast. Ray (2004) morphologically described *Saccharomyces* cells as spheroidal, cylindrical or elongated with approximate 5-30 x 2-10 μm of size. On agar, their colonies appear with white or creamed colour and typical yeast odor. In *Saccharomyces* genus, *S. ellipsoideus* is responsible for wine making; while *S. cerevisiae*, derived from wild yeast used for manufacture beer and wine a long time ago, has been still widely applied in brewing, wine and even bakery industry. Indeed, there have been several studies in which *S. cerevisiae* was used for alcoholic fermentation, especially as such a substrate for vinegar production (Horiuchi *et al*., 1999; Vithlani and Patel, 2010).

2.5.2 Mechanisms of alcoholic fermentation

Alcoholic fermentation is a biochemical process relates to oxidation sugary substrate into ethanol by action of certain microorganisms, especially yeast. In general, glucose is the major carbon source for energy production in yeast. Yet, it

Prince of Songkla University Pattani Campus

also can utilize a number of non-conventional carbon sources, such as biopolymers, pentoses, alcohols, polyols, hydrocarbons, fatty acids and organic acids which are preliminarily converted into simple constituents for consumption by their enzymes. For example, disaccharides, such as maltose, sucrose, melibiose, lactose or cellobiose can easily be accepted as nutrients by the actions of corresponding hydrolases which break these disaccharides down into their constituent mono-saccharides (Rodrigues *et al*., 2006).

Principally, glucose is the major source that yeast consumes in glycolysis pathway with pyruvate formed at the end of process. Glycolysis is a catabolic process, whereby production of energy in form of Adenosine triphophate (ATP) is coupled to the generation of the intermediates (i.e. pyruvate) and the reduced form of nicotinamide adenine dinucleotide (NADH) for biosynthetic pathways. Two principal modes of the use of pyruvate in further energy production can be distinguished: respiration and fermentation.

The respiration is a complex process that begins with glycolysis, followed by the Krebs cycle and the electron transport chain in the presence of oxygen. The pyruvate produced from previous glycolysis enters the mitochondrial matrix to be oxidatively decarboxylated. Acetyl CoA is the consequent metabolite that initiate citric acid cycle to generate two molecules of CO_2 and chemically reductive energy in form of NADH, $FADH_2$ (reduced form of Flavin Adenine Dinucleotide) and ATP. The large amount of chemical energy stored in previous reductive energy is released in the subsequent electron transport chain process. The released energy is captured in the form of ATP by oxidative phosphorylation with oxygen as the electron acceptor. Most of this ATP is produced by the electron transport chain which can only function if O_2 is available.

Figure 4 particularly shows the fermentation process performing with the sugar metabolism in yeast. In alcoholic fermentation of sugars, pyruvate from glycolysis first is decarboxylated by pyruvate decarboxylase into acetaldehyde, and CO_2 released in this process. The acetaldehyde is then reduced by NADH to ethanol (ethyl alcohol) with activity of alcohol dehydrogenase (AlDH) enzyme.

Prince of Songkla University Pattani Campus

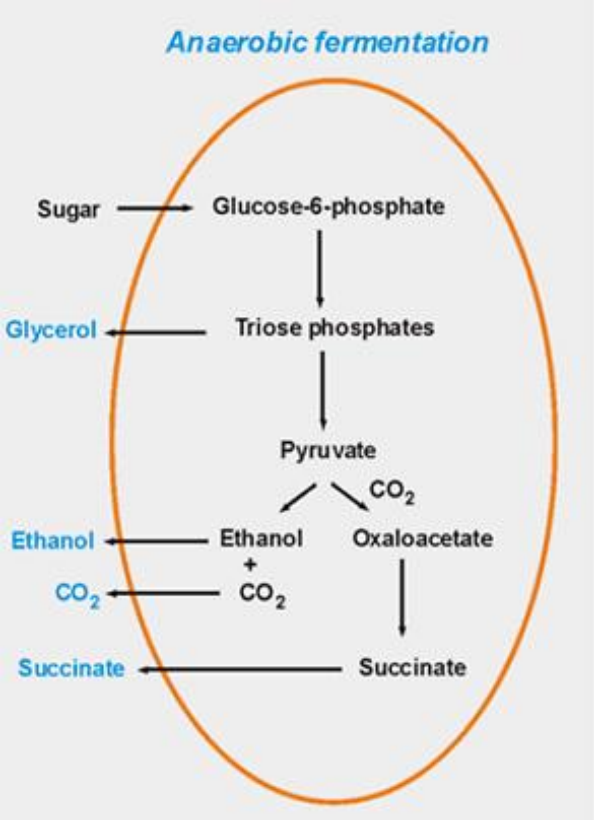

Figure 4 The metabolism of fermentation in yeast

Source: Walker (1998)

However, fermentation does not necessarily have to be carried out in an anaerobic environment. For example, even in the presence of abundant oxygen, yeast cells greatly prefer fermentation to oxidative phosphorylation, as long as sugars are readily available for consumption. This phenomenon is known as the Crabtree effect, which has been explained from a limited respiratory capacity of respiratory sugar metabolism (Rodrigues *et al*., 2006).

2.5.3 Factors affect to alcoholic fermentation

2.5.3.1 Oxygen

Most yeasts preliminarily require an abundance of oxygen for growth, but the controlling the supply of oxygen later is required for alcoholic fermentation in anaerobic condition. Alcoholic fermentation in anaerobic condition produced higher ethanol content within longer time to complete as compared to fermentation in aerobic condition. Indeed, Ciani *et al*. (2000) showed the fermentation of *S. cerevisiae* DBVPG 6663 in both anaerobic and aerobic conditions with ethanol contents of 105.9 g/l and 50.7 g/l, respectively.

Giovanelli *et al*. (1996) pointed out that 100 hours were needed to complete the aerobic fermentation of grape juice, while sugars were exhausted within 200 hours under anaerobic conditions. Similarly, the significantly higher ethanol content was observed in anaerobic condition as compared with aerobic condition upon activity of *S. cerevisiae* TISTR 5049. On the other hand, the biomass produced in aerobic condition was higher than the one of counterpart (Wongsudaluck, 2012).

2.5.3.2 Sugar content

For alcoholic fermentation, substrate concentration (i.e. sugar content) is an important factor in fermentation. Kumoro *et al*. (2012) found out that upon activity of baker's yeast, the increase in sugar content from 8 to 14% increased the ethanol produced and remained the higher residual sugar content within longer time to complete fermentation. In study on thick juice fermentation, substrate concentrations from 5.0 to 15.0 g/L also showed the significantly positive effects on the ethanol produced (Grahovac *et al*., 2012).

However, the decrease in ethanol production, even the yeast cells, at the high sugar concentration has been mentioned by several researchers. For examples, substrate concentrations from 200 g/L to 300 g/L gave the growth rate decline for some yeasts and final cell biomass (Charoenchai *et al*., 1998). The high glucose concentrations up to 300 kg/m^3 decreased the ethanol conversion efficiency that explained by the internal pH changes as by-products accumulated and ethanol formed (Lin *et al*., 2012). Besides, the high substrate concentration additionally might cause the osmotic shock that affected directly on the rate of glycolysis, resulted in the death of cells (Nagodawithana *et al*., 1974).

2.5.3.3 Microbial concentration

The yeast concentration played an important role for fermentation performance, especially influences on fermentation time. Pereira *et al.* (2013) showed that in high-cell-density fermentation of honey-must, the more cells were applied (up to 10^8 cfu/ml), the higher consumption rate was observed in both yeasts (QA3 and ICV D47). In terms of using baker's yeast for fermentation, Ocloo and Ayernor (2010) completely fermented 3000 mL cassava flour hydrolysate to attain 8.30±0.70 (%) ethanol concentration after 120 hours, 72 hours and 48 hours with 100 mL of

Prince of Songkla University Pattani Campus

20.0, 15.0 and 5.0 % (v/v) baker's yeast, correspondingly approximated to 0.65, 0.5 and 0.1% (w/v) baker's yeast, respectively.

Kumoro *et al*. (2012) fermented clarified jackfruit juice of 14 % (w/w) initial sugar with different (*Saccharomyces cerevisiae*) concentrations 0.5, 1.0, 1.5 and 2.0 % (w/v) under anaerobic condition at 30°C for 14 days. The increase in baker's yeast concentration from 0.5 to 1.0% (w/v) obviously increased the fermentation rate, but the insignificant difference was not observed at the concentrations from 1.0% (w/v) upwards. As the results, approximately 12.13% (v/v) ethanol attained after 9 days of fermentation with 0.5 %(w/v) baker's yeast, while only 7 days were sufficient to obtain the similar ethanol content at the higher concentrations of baker's yeast.

Akin-Osanaiye (2008) reported that the pawpaw fruit waste performed alcoholic fermentation within 3 days attained 3.04±0.08; 3.30±0.03, 3.28±0.01 and 3.34±0.03 ethanol contents at 1, 2, 5 and 10% baker's yeast.

2.6 Acetous fermentation

2.6.1 Acetic acid bacteria

In terms of morphology, Guillamón and Mas (2009) described acetic acid bacteria with ellipsoid or cylindrical shape, which belong to gram-negative or gram-variable microbes. They could be seen under microscopy in individuals, pairs or in clumps with 0.4-1.0μm in width and 0.8 - 4.5μm in length. Acetic acid bacteria belong to obligated aerobic group which able to archive oxidation reactions in presence of oxygen as a terminal electron acceptor. They have flagella for them to be motile, but they do not form endospores as a defensive resistance. The optimum pH for the growth of acetic acid bacteria is 5-6.5, yet even in low pH 3.0-4.0 they still can grow.

Acetic acid bacteria are available in sugary, alcoholised or slightly acid substrates (e.g. fruits, beer, wine, cider) and enable to oxidize substrates into organic acids as final products. Certainly, acetic acid bacteria can oxidize a wide variety of substrates such as carbohydrates, organic acids, and nitrogen. Particularly, when the substrate is ethanol, acetic acid is produced to make vinegar, and this is where the name of the bacterial group comes from. In vinegar, *Acetobacter* (*A. aceti*) and

Prince of Songkla University Pattani Campus

Gluconobacter (*G. suboxydans*) genera are mostly available and well-known as the acetic acid bacteria produced vinegar (Lasko *et al*., 1997). With the ability to produce more acetic acid, *Acetobacter* genus is favorable for vinegar production (Solieri and Giudici, 2009). Hutkins (2008) reported that *Acetobacter aceti* is a primary organism which has been extensively applied in vinegar manufacture. Indeed, there have been several studies using *Acetobacter aceti* to produce vinegar (Horiuchi *et al*., 1999; Vithlani and Patel, 2010; Kocher *et al*., 2014).

2.6.2 Mechanisms of acetous fermentation

Acetic acid fermentation related to biochemical and physiological processes occur within the periplasmic space and cytoplasmic membrane of obligate aerobes, instead of in cytoplasm. Thereafter, energy is not yielded by the substrate level phosphorylation in normal catabolic pathways. In contrast, the electron transport and oxidative phosphorylation reaction in respiratory chain are responsible to generate ATP as the yield. The mechanism of acetification from ethanol into acetic acid is illustrated in Figure 5.

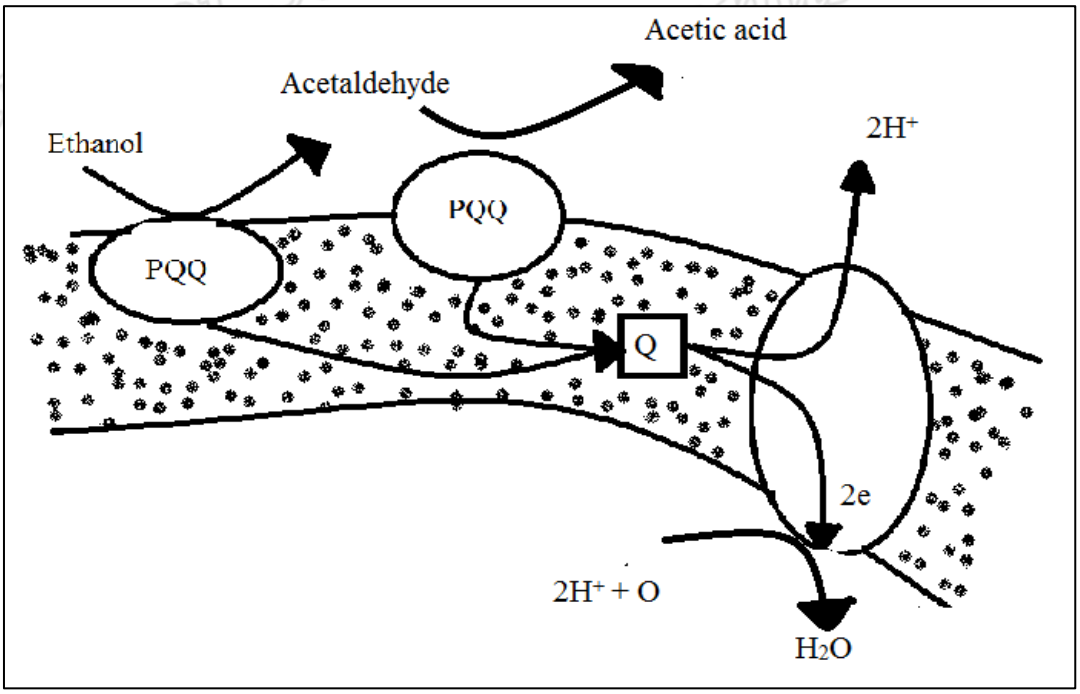

Figure 5 The oxidation of ethanol into acetic acid in *Acetobacter* bacteria
Source: Adam (1998)

Hutkins (2008) described that two enzymatic systems including alcohol dehydrogenase (AlDH) and acetyldehyde dehydrogenase (AcDH) for the conversion of ethanol to acetaldehyde in acetic acid bacteria. AlDH is linked to the cytoplasmic membrane, while AcDH is in the cytoplasm. They contain pyrroloquinoline quinone (PQQ) which has specific activity as electron acceptor for oxidation of alcohol into acetaldehyde and from acetaldehyde to acetic acid. Ethanol dehydrogenase in *Acetobacter* genus was reported to be more stable than the one from *Gluconobacter* genus, resulted in more complete oxidation for vinegar production. Electrons production from the oxidative reactions is transferred by these enzymes into the cytochromes for respiratory chain. Eventually, energy is generated by physiological processes in respiratory chain for microbial growth and development with acetic acid and water as the final products. Some acetic acid bacteria such as *Acetobacter aceti*, *Acetobacter pasteurianus* and *Gluconobacte*r (belongs to *suboxydans* group) have ability to oxidize further acetic acid into CO_2 and H_2O in absence of ethanol (Macauley *et al*., 2001). In actual vinegar production, this phenomenon causes disappearance of acetic acid, which is undesirable and called over-oxidation (Solieri and Giudici, 2009).

2.6.3 Factors affect to the acetous fermentation

2.6.3.1 Oxygen and agitation (shaking speed)

Acetic acid bacteria belong to obligated aerobes microorganism with high oxygen requirement for metabolism. The presence of oxygen could maintain intracellular ATP of bacteria, which is necessary to prevent the cells from penetration of acetic acid. As the result, the cells are not broken down and the fermentation is kept going on (Schlepütz *et al*., 2013).

Tesfaye *et al*. (2000) stated that stirring or agitation facilitated the oxygen transfer from the gas phase to the liquid phase by breaking the large bubbles at the entrance of air. In their research, with 450 rpm and 250 rpm for agitation speed, fermentation performed with higher efficiency and 450 rpm was reported as the optimal stirring speed.

Schlepütz *et al*. (2013) referred the results with 30 seconds for oxygen interruption caused reduction of 17 % for acetic acid formation rate, in spite of the high ethanol concentration (65g/L) and at the early phase of fermentation.

Prince of Songkla University Pattani Campus

Kocher *et al*. (2014) reported that fermentation with agitation at 75 rpm could reduce the time as one-half as the time of control sample (sample without agitation), while acetic acid contents corresponding are 3.99 and 2.14 (w/v).

2.6.3.2 Ethanol content

Ethanol is the main carbon source for acetic acid bacteria in vinegar production. However, at higher concentrations, their growth could be inhibited. The ethanol concentration could be varied from 2%-10% (v/v) for fermentation substrate. Wongsudaluk (2012) showed the growth of 6% ethanol was better than the one of 8% ethanol. According to Gullo *et al.* (2006), fermented cooked must with less than 8% (v/v) of ethanol was commonly applied in traditional balsamic vinegar.

2.6.3.3 Nutrients

The supplementation of nutrients like growth factors and minerals enhance the fermentation efficiency as compared with the fermentation. According to result of Kocher *et al*. (2014), approximate 24 days were necessary to attain approximate 5% acidity in static condition without any supplementation. Otherwise, with nutrients and shaking the fermentation time was reduced to about one-third as compared with the former. From that, the acetic acid contents were corresponding higher in supplement treatment with 6.10% (w/v) acidity.

Prince of Songkla University Pattani Campus

CHAPTER 3
MATERIALS AND METHODS

3.1 Microorganisms

3.1.1 Acetic acid bacteria

Acetobacter aceti TISTR 102 strain was bought from the Thailand Institute of Scientific and Technological Research was inoculated in GYC agar (D-glucose, 100g/L; yeast extract, 10g/L; agar, 20g/L and calcium carbonate 10g/L). The culture was incubated at 30^{o}C for 24 hrs, subsequently stored at 4^{o}C and subcultured every month to preserve the pure culture.

3.1.2 Commercial baker's yeast

Commercial baker yeast (Angel Yeast Gold Baker's) was bought from the local market in Pattani province (Thailand) was used in this research. The cell viability of baker's yeast was determined by spread plate technique on YEPD agar (D-glucose, 100g/L; yeast extract, 10g/L; peptone, 10g/L and agar, 20g/L) and expressed as colony forming unit per gram powder (CFU/g). The viable cell concentration of baker's yeast was 2.1x10^{9} CFU/g. The commercial baker's yeast in instant dry form is shown in Figure 24 (Appendix C).

3.2 Experiments

3.2.1 Properties determination of coconut water and banana juice

3.2.1.1 Coconut water

Coconut water was collected from the coconut milk store in Pattani province (Thailand). Then, it was filtered through a thin cotton cloth and boiled in 10 minutes for pasteurization before storage in deep-freezing condition (-20^{o}C).

To analyze physiochemical properties of coconut water, the pH value and total soluble solid were recorded by a digital pH meter (Metler Toledo, SevenEasy S-20K) and a hand-refractometer (Atago N1), respectively. Total sugar and reducing sugar were determined by phenol sulfuric acid method (Dubois *et al*., 1956) and DNS method (Miller, 1959), respectively. The Kjeldahl method (AOAC, 2000) was applied to estimate total nitrogen. Phosphorous, sulfur, potassium, sodium, calcium, magnesium, zinc, copper and iron were estimated with atomic absorption spectrometry (AOAC, 2000).

Prince of Songkla University Pattani Campus

3.2.1.2 Banana juice

Namwa bananas (*Musa sapientum* L.), which were still slightly green and bought in the local market in Pattani province (Thailand), were naturally ripe at ambient temperature. The bananas that had yellow colour with slightly brown spot on the peel were used for the research and shown in Figure 21 (Appendix C).

The bananas were washed, peeled and chopped into small pieces before adding hot water at ratio 1 water: 1 banana pulp. The banana mash was heated in water bath at 95^{o}C for 1 hr and then was filtered through a thin cloth to obtain the banana juice. Banana juice was boiled in 10 minutes at 75^{o}C for pasteurization before storage in deep-freezing condition (-20^{o}C).

To analyze physiochemical properties of banana juice, the pH value and total soluble solid were recorded by a digital pH meter (Metler Toledo, SevenEasy S-20K) and a hand-refractometer (Atago N1), respectively. Total sugar and reducing sugar were determined by phenol sulfuric acid method (Dubois *et al*., 1956) and DNS method (Miller, 1959), respectively. The Kjeldahl method (AOAC, 2000) was applied to estimate total nitrogen. Phosphorous, sulfur, potassium, sodium, calcium, magnesium, zinc, copper and iron were estimated with atomic absorption spectrometry (AOAC, 2000).

3.2.2 Optimization of culture conditions for *A. aceti* TISTR 102

3.2.2.1 Effect of banana juice volume on the bacterial growth

Coconut water was standardized to approximately 10% (w/v) total sugar by commercial sucrose (Milk Phor), subsequently was supplemented with banana juice at different ratios: 0, 25, 50, 75 and 100% (v/v). The GY medium was used as the reference medium. All experimental culture media were adjusted to pH 5.0±0.05 by either HCl 0.1N or NaOH 0.1N. The 500 mL-round bottom flasks contained 300 mL working volume were autoclaved at 121^{o}C for 15 minutes.

A full loop of *A. aceti* TISTR 102 from GYC agar plate was inoculated into GY broth (D-glucose, 100g/L; yeast extract, 10 g/L). The culture was incubated on rotary shaker at 30ºC and 120 rpm within 18-24 hrs to measure optical density at 600 nm absorbance (OD_{600}). The OD_{600} was adjusted to 0.5±0.05 before inoculating into experimental media. Starter culture at 5% (v/v) was inoculated into experimental media and incubated in rotatory shaker for 72 hrs at 30^{o}C and 120 rpm. Sampling was

Prince of Songkla University Pattani Campus

carried out at every 6 hrs in the first 48 hrs and every 12 hrs for the consequent time. Samples were taken out to determine pH, reducing sugar by DNS method (Miller. 1959) and viable cells by spread plate technique on GYC agar. The incubation conditions were 30^{o}C for 24-48 hrs. The cell viability was considered as the predominant criterion to assess the effectiveness of experimental medium.

Completely randomized design (CRD) was applied in this experiment with 3 replications. The collected data were analyzed based on ANOVA and presented as mean values with standard deviations. Significant differences within the treatments were analyzed by Duncan's multiple range test (DMRT) at a 5% probability level ($p \leq 0.05$)

3.2.2.2 Effect of ammonium sulfate on the bacterial growth

Coconut water was standardized to approximately 10% (w/v) total sugar by sucrose (Milk Phor), subsequently was supplemented with 50 % (v/v) banana juice for culturing *A. aceti* TISTR 102. Ammonium sulfate was varied at different concentrations of 0 (control), 0.1, 0.3 and 0.5% (w/v) to supplement into the culture medium. All experimental media were adjusted to pH 5.0±0.05 by either HCl 0.1N or NaOH 0.1N. The 500 mL-round bottom flasks contained 300 mL working volume were autoclaved at 121^{o}C for 15 minutes.

A full loop of *A. aceti* TISTR 102 from GYC agar plate was inoculated into GY broth (D-glucose, 100g/L; yeast extract, 10 g/L). The culture was incubated on rotary shaker at 30ºC and 120 rpm within 18-24 hrs to measure optical density at 600 nm absorbance (OD_{600}). The OD_{600} was adjusted to 0.5±0.05 before inoculating into experimental media. Starter culture at 5% (v/v) was inoculated into experimental media and incubated in rotatory shaker for 72 hrs at 30^{o}C and 120 rpm. Sampling was carried out at every 6 hrs in the first 48 hrs and every 12 hrs for the consequent time. Samples were taken out to determine pH, reducing sugar by DNS method (Miller, 1959) and viable cells by spread plate technique on GYC agar. The incubation conditions were 30^{o}C for 24-48 hrs. The cell viability was considered as the predominant criterion to assess the effectiveness of experimental medium.

Completely randomized design (CRD) was applied for study on effects of ammonium sulfate. The collected data were analyzed based on ANOVA and presented as mean values with standard deviations. Significant differences within the

treatments were analyzed by Duncan's multiple range test (DMRT) at a 5% probability level ($p \leq 0.05$)

3.2.2.3 Effect of yeast extract on the bacterial growth

Coconut water was standardized to approximately 10% (w/v) total sugar by sucrose (Milk Phor), subsequently was supplemented with 50 % (v/v) banana juice for culturing *A. aceti* TISTR 102. Yeast extract was varied at different concentrations of 0 (control), 0.2%, 0.6% and 1.0% (w/v) to supplement into the culture medium. All experimental media were adjusted to pH 5.0±0.05 by either HCl 0.1N or NaOH 0.1N. The 500 mL-round bottom flasks contained 300 mL working volume were autoclaved at 121^{o}C for 15 minutes.

A full loop of *A. aceti* TISTR 102 from GYC agar plate was inoculated into GY broth (D-glucose, 100g/L; yeast extract, 10 g/L). The culture was incubated on rotary shaker at 30^{o}C and 120 rpm within 18-24 hrs to measure optical density at 600 nm absorbance (OD_{600}). The OD_{600} was adjusted to 0.5±0.05 before inoculating into experimental media. Starter culture at 5% (v/v) was inoculated into experimental media and incubated in rotatory shaker for 72 hrs at 30^{o}C and 120 rpm. Sampling was carried out at every 6 hrs in the first 48 hrs and every 12 hrs for consequent time. Samples were taken out to determine pH, reducing sugar by DNS method (Miller, 1959) and viable cells by spread plate technique on GYC agar. The incubation conditions were 30^{o}C for 24-48 hrs. The cell viability was considered as the predominant criterion to assess the effectiveness of experimental medium.

Completely randomized design (CRD) was individually applied for study on effects of yeast extract. The collected data were analyzed based on ANOVA and presented as mean values with standard deviations. Significant differences within the treatments were analyzed by Duncan's multiple range test (DMRT) at a 5% probability level ($p \leq 0.05$)

3.2.2.4 Effect of shaking speed on the bacterial growth

Coconut water at 10% (w/v) total sugar and 50 % (v/v) added banana juice without yeast extract and ammonium sulfate supplementation was applied for culturing *A. aceti* TISTR 102. The 500 mL-round bottom flasks contained 300 mL of working volume with adjusted pH 5.0±0.05 were autoclaved at 121^{o}C for 15 minutes

Prince of Songkla University Pattani Campus

A full loop of *A. aceti* TISTR 102 from GYC agar plate was inoculated into GY broth (D-glucose, 100g/L; yeast extract, 10 g/L). The culture was incubated on rotary shaker at 30ºC and 120 rpm within 18-24 hrs to measure optical density at 600 nm absorbance (OD_{600}). The OD_{600} was adjusted to 0.5±0.05 before inoculating into experimental media. Starter culture at 5% (v/v) was inoculated into culture media and incubated in rotatory shaker for 72 hrs at 30^{o}C. Shaking speed was varied into 120 and 150 rpm. Sampling was carried out at every 6 hrs in the first 48 hrs and every 12 hrs for consequent time. Samples were taken out to determine pH, reducing sugar by DNS method and viable cells by spread plate technique on GYC agar. The incubation conditions were 30^{o}C for 24-48 hrs. The cell viability was considered as the predominant criterion to assess the effectiveness of experimental medium

Completely randomized design (CRD) was applied in this experiment with 3 replications and data were presented as mean values with standard deviations. Data were analyzed based on ANOVA - *t*-test at a 5% probability level ($p \leq 0.05$).

3.2.3 Preparation and storage of *A. aceti* TISTR 102 starter powder.

3.2.3.1 Effect of sucrose concentration on bacterial cells in drying

The study on sucrose as a thermal protectant was carried out with concentrations 10, 20 and 30% (w/v). Sucrose solution was prepared with distilled water and commercial sucrose (Milk Phor). *A. aceti* TISTR 102 was cultured in coconut water with 50% (v/v) added banana juice within 21 hrs, 30ºC and 150 rpm. The pellet (cells) was collected by centrifugation with 9,600g at 4ºC for 10 minutes and purified with phosphate buffer 50 mM 6.5 pH two times. The purified cells were adjusted to concentration of 10^{8}-10^{9} CFU/mL by mixing the cells from 5 eppendoft tubes and subsequently were resuspended in distilled water (control), 10, 20 and 30% (w/v) sucrose solutions within 1 hr. The cell suspensions consequently were mixed with sterilized rice bran with ratio 4mL: 10g and dried at 35^{o}C for 12 hours. The rice bran initially contained 8.24±0.02 (%) moisture content, while the rice bran was autoclaved at 121^{o}C for 15 minutes contained 10.51±0.08 (%) moisture content. The rice bran before and after autoclaving are shown in Figure 23 (Appendix C).

Starter culture was rehydrated in sterilized distilled water to determine the viable cells by spread plate technique on GYC agar. The results of cell viability were expressed as log CFU/g. The ratio of the cell viability in starter powder between

Prince of Songkla University Pattani Campus

before and after drying was residual percentage - the predominant criterion to determine effect of protectant in thermal drying. The moisture content was evaluated according to AOAC (2000); while water activity was measured with water activity meter (AQUA LAB 3TE).

Completely randomized design (CRD) was applied in this experiment with 3 replications. The collected data were analyzed based on ANOVA and presented as mean values with standard deviations. Significant differences within the treatments were analyzed by Duncan's multiple range test (DMRT) at a 5% probability level ($p \leq 0.05$).

3.2.3.2 Effect of silica gel on starter powder during storage

Approximate 10g of starter powder in aluminum foil pouch (6cm×10cm) were packed and sealed with vacuum condition. The starter powder packages then were covered with PE plastic bag (10cm×15cm) in presence of silica gel (SILICA GEL WHITE–Tippayakiat Co., Ltd) (Figure 22). The packages without silica gel were the control samples. Starter powder was stored at 4^{o}C in refrigerator within 2 months. The cell availability, moisture content and water activity were monitored every 10 days. The starter powder was rehydrated in sterilized distilled water and serially diluted to determine the viable cells by spread plate technique on GYC agar. The moisture content is evaluated according to AOAC (2000), while water activity is measured with water activity meter (LAB AQUA).

Completely randomized design (CRD) was applied in this experiment with 3 replications. The collected data were analyzed based on ANOVA and presented as mean values with standard deviations. For each treatment, significant differences within the interval sampling times were analyzed by Duncan's multiple range test (DMRT) at a 5% probability level ($p \leq 0.05$).

3.2.3 Preparation of vinegar from coconut water using baker's yeast and *A. aceti* TISTR 102 starter powder

3.2.3.1 Alcoholic fermentation: Effect of baker's yeast concentration

Coconut water standardized into 14% (w/v) sugar content with commercial sucrose (Milk Phor) was boiled in 10 minutes for pasteurization. The pasteurized fermenting liquid then was transferred in 2,000 mL round bottom flask with approximately 1,500 mL working volume. The commercial baker's yeast (Angel

Prince of Songkla University Pattani Campus

Yeast Gold Baker's) was in instant dry form and contained $2.1x10^9$ CFU/g. Baker's yeast concentrations of 0.2, 0.4 and 0.6% (w/v) that corresponded to $6.3x10^9$, $1.3x10^{10}$ and $1.9x10^{10}$ CFU/g of yeast cell concentration, respectively, were inoculated directly into fermenting liquid. Fermentation process performed in round bottom flask fitted with cotton at room temperature (30±2°C) in 7 days at static condition. The sample was carried out daily to determine some specific properties. The pH and total soluble solid were monitored using a digital pH meter and refractometer, respectively. The total sugar was measured by phenol sulfuric acid method (Dubois *et al*., 1956), while ethanol concentration results were obtained from Ebulliometer equipment (Wongsudaluk, 2012)

Completely randomized design (CRD) was applied in this experiment with 3 replications. The collected data were analyzed based on one factor ANOVA and presented as mean values with standard deviations. Significant differences within the treatments were analyzed by Duncan's multiple range test (DMRT) at a 5% probability level ($p \leq 0.05$).

3.2.3.2 Alcoholic fermentation: Effect of sugar concentration

Coconut water was adjusted into various sugar concentrations 12, 16 and 20% (w/v) by commercial sucrose (Milk Phor) and then boiled in 10 minutes for pasteurization. The pasteurized fermenting liquid then was transferred in 2,000 mL round bottom flask with approximately 1,500 mL working volume. The baker's yeast (Angel Yeast Gold Baker's) at 0.4% (w/v) concentration was inoculated directly into fermenting liquid in round bottom flask fitted with cotton. The alcoholic fermentation performed at room temperature (30±2°C) for 7 days at static condition. Sampling was carried out daily to determine ethanol content, total sugar content, pH and total soluble solid. pH meter and refractometer were used for monitoring the pH and total soluble solid. Phenol sulfuric acid method were applied to measure total sugar (Dubois *et al*., 1956) and ethanol concentration results were obtained from Ebulliometer equipment (Wongsudaluk, 2012)

Completely randomized design (CRD) was applied in this experiment with 3 replications. The collected data were analyzed based on one factor ANOVA and presented as mean values with standard deviations. Significant differences within

the treatments were analyzed by Duncan's multiple range test (DMRT) at a 5% probability level ($p \leq 0.05$).

3.2.3.3 Acetification: Effect of *A. aceti* TISTR 102 starter powder

Coconut wine attained approximately 6% ethanol was subsequently applied for acidification process at different *A. aceti* TISTR 102 starter powder concentrations. The starter powder contained $1.3x10^7$ CFU/g with 0.480±0.008 water activity and 7.17±0.17 % moisture content. The starter powder concentrations of 0.5, 1.0, 1.5 and 2.0% (w/v) that corresponded to $9.8x10^7$, $1.9x10^8$, $2.9x10^8$ and $3.9x10^8$ CFU/g of bacterial cell concentration were added directly into 1,500 mL coconut wine in 2,000 mL round bottom flask fitted with cotton. The fermentation performed at room temperature (30±2^{o}C) within 30 days at static condition and sampling was monitored every 3 days. The pH was checked by a digital pH meter. Acidity was measured by titration with NaOH 0.1N (AOAC, 2000) and expressed in terms of acetic acid, while ethanol concentration results were obtained from Ebulliometer. The fermentation efficiency (FE) of the optimal treatment was calculated as follow

$$FE = \frac{\%\ Acidity\ (\frac{w}{v})}{\%\ Ethanol\ (\frac{v}{v})\ x\ 1.043} x100$$

Completely randomized design (CRD) was applied in this experiment with 3 replications. The collected data were analyzed based on one factor ANOVA and presented as mean values with standard deviations. Significant differences within the treatments were analyzed by Duncan's multiple range test (DMRT) at a 5% probability level ($p \leq 0.05$).

3.2.3.4 Preliminary quality evaluation of coconut vinegar

Coconut water was fermented into vinegar with baker's yeast and *A. aceti* TISTR 102 starter powder. The palm sap vinegar traditionally fermented within 3 months was bought in the market in Pattani province (Thailand) was used as the control sample. The qualities of two vinegars including acidity, residual ethanol, total soluble solid and pH were determined. The results were evaluated with 3 replications and expressed as mean ± standard deviation.

Sensory evaluation test for only appearance (i.e. colour and turbidity), odor, flavor and overall acceptance was carried out with 9-point hedonic scale with 30

Prince of Songkla University Pattani Campus

panelists. Samples were served in clean transparent glasses (tumblers) which had been labeled with 3-digit random numbers. Questionnaires and water for mouth rinsing between each tasting were provided. Prior to evaluation, the panelists were asked to read through the questionnaires, and the meaning of each attribute (appearance, odor and sourness) was explained to the panelists to avoid any misinterpretation. The scoring used was 1-dislike extremely; 2-dislike very much; 3-dislike; moderately; 4-dislike slightly; 5-neither like nor dislike; 6-like slightly; 7-like moderately; 8-like very much; 9-like extremely. The sensory evaluation form is attached in Appendix B, while the two samples are illustrated in Figure 24 (Appendix C).

The scores were obtained from 30 panelists for each sensorial attribute and expressed as mean ± standard deviation. The *t*- test was used for determining the significant difference between two samples with the significantly statistical level

3.3 Chemicals and Equipment

3.3.1 Chemicals

1. Agar (Himedia)
2. D-glucose (Arjax)
3. Calcium carbonate ($CaCO_3$, Merck)
4. Yeast extract powder (Himedia)
5. Peptone (Merck)
6. Crystal phenol (Pancera)
7. Hydrochloric acid (HCl, Lab Scan)
8. Potassium hydrogen phthalate ($KHC_8H_4O_4$)
9. Phenolphtalein (Carlo ERGA)
10. Sodium potassium tartrate ($NaKC_4H_4O_6$, Ajax)
11. Mono-sodium phosphate ($NaH_2PO_4.2H_2O$, Ajax)
12. Di-sodium phosphate ($Na_2HPO_4.2H_2O$, Ajax)
13. 3,5-Dinitrosalicylic acid (Aldrich)
14. Sodium carbonate ($NaCO_3$, Arjax)
15. Sodium hydroxide (NaOH, Merck)
16. Sulfuric acid (H_2SO_4, Lab Scan)
17. Ammonium sulfate ($(NH_4)_2SO_4$, Lab Scan)

Prince of Songkla University Pattani Campus

18. Boric acid (H_3BO_3, Ajax)
19. 99% Ethanol (C_2H_5OH, Merck)

3.3.2 Glassware

1. Beaker 100 mL, 250 mL, 400 mL, 600 mL and 1,000 mL
2. Biuret 25 mL (Weteg)
3. Laboratory bottle 250 mL and 500 mL (Schott)
4. Cylinder 100 mL, 500 mL and 1,000 mL
5. Erlenmeyer flask 250 mL, 500 mL and 1,000 mL
6. Micro pipette 100 µl, 1 ml and 5 mL
7. Petri disk
8. Pipette 1mL and 10mL
9. Round bottom flask 500 mL, 1,000 mL
10. Spreader and loop

3.3.3 Equipment

1. Centrifuge (Hettich Zencrifuhen ROTINA 420R)
2. Oven (Memmert, 600)
3. Autoclave (HIRAYAMA)
4. Refrigerator (SANYO)
5. Shaker (Chil Tern scienctific , Orbital shaker SS70)
6. Water activity meter (AQUA LAB 3TE)
7. pH meter (Schott Instrument, Lab 850)
8. Incubator (B. Braun Biotech International)
9. Vortex mixer (Scientific Industries, Vortex Genie 2)
10. Hand refractometer (Atago N1)
11. Thermometer (TEL-TRU)
12. Spectrophotometer (Biochom Libra S22)
13. Balance (Metler Toledo TG 5002-S)
14. Ebulliometer (UJARDIN-SALLERON)
15. Laminar Flow (FM 14-09-01)
16. Moisture balance (Satorius MA 35)
17. Kjeldahl Apparatus (Gerhardt)

Prince of Songkla University Pattani Campus

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Approximately physiochemical properties of coconut water and banana juice

The approximate physiochemical properties of coconut and banana juice are presented in Table 5. In coconut water, the total soluble solid, pH, total sugar and nitrogen were 4.23±0.25, 5.42±0.03, 2.61±0.13 (g/100mL) and 1.20±0.10 (mg/mL), respectively that consistent with several previous studies. For examples, total soluble solid was 4.0±0.2 ^{o}Brix and nitrogen content was approximately 10 mg/L (Unagul *et al*., 2007). The pH was 5.36±0.00 and total sugar was 2.03±0.01 (Supakod and Wongwicharn, 2012). In the research, the amount of reducing sugar was 1.85±0.08 g/100mL, occupied more than 50% (w/w) of total available sugar in coconut water. In term of microbiology, carbon source such as sugar was essential to form carbon-containing-substances, for example protein, fats, carbohydrates or lipids. The macronutrients, including calcium, magnesium, potassium, sodium and phosphorus revealed to the cellular composition and inner enzymatic reactions were available with noticeable amounts: 82.30±4.17, 55.80±1.52, 1017.40±23.74, 97.00±0.5 and 90.00±0.00 (mg/L), respectively. Tan *et al* (2013) studied on physiochemical changes of coconut water in Malaysia within different mature stages and they reported that most of minerals contained in overly-mature coconut were remarkably available. For examples, calcium, magnesium, potassium and sodium were 23.98±0.054, 31.65±0.038, 35.11±0.133 and 36.51±0.020 (mg/100mL), respectively. The trace elements such as zinc, copper and iron were not mentioned in previous studies, but they were available in coconut water with small amounts 0.22±0.04, 0.03±0.004 and 0.07±0.001 (mg/L).

For banana juice, a turbid and slightly purple juice was extracted from the pulp, which consequently became darker with cloudy precipitate formation after sterilization in the autoclave. Generally, most of nutrients determined in extracted banana juice were significantly available as compared to coconut water. Indeed, the major nutrients such as magnesium, potassium and phosphorus were presented with considerably high amounts: 138.90±1.84; 1604.40±21.28 and 330±0.00 (mg/L). But, calcium and sodium were present with 16.20±0.35 and 20.10±0.14 (mg/L) that less

than amounts contained in coconut water. The trace elements, including zinc, copper and iron act as cofactors for essential enzymatic reactions in the cell were noticeably available with 1.02±0.06; 0.11±0.01 and 1.52±0.08 (mg/L), respectively. The pH value was recorded with 4.44±0.02, while pH 4.25±0.01 was found in enzyme-extracted banana juice from namwa banana (e.g. *Musa sapientum* L.) (Supakod and Wongwicharn, 2012). The total soluble solid was 14.1±0.10 ºBrix; the total sugar and reducing sugar were remarkably 10.70±0.13 and 8.86±0.13 (g/100mL), respectively. The nitrogen known as required substance for the synthesis of proteins, amino acids, DNA and RNA was available with 2.70±0.3 mg/100 mL and was twice as much as the nitrogen in coconut water.

With the high nutrients, coconut water has been used as substrate or additive in biotechnological application (Unagul *et al*., 2007) or microbial culturing (Supakod and Wongwicharn, 2012). Additionally, it could not be denied the nutrients in banana juice with the higher quantities, leading to be potentially used in several applications as coconut water was (Singh *et al*., 2005). Besides, banana juice also possibly combines to coconut water as economically efficient medium for acetic acid bacteria culture (Supakod and Wongwicharn, 2012).

Table 5 Approximately physiochemical properties of coconut water and banana juice

Properties & Compositions	**Coconut water**	**Banana juice**
Total soluble solid (ºBrix)	4.23±0.25	14.1±0.10
pH	5.42±0.03	4.44±0.02
Total sugar (% or g/100 mL)	2.61±0.13	10.70±0.13
Reducing sugar (% or g/100mL)	1.85±0.08	8.86±0.13
Nitrogen (mg/100 mL)	1.20±0.10	2.70±0.30
Minerals (mg/L)		
Calcium	82.30±4.17	16.20±0.35
Magnesium	55.80±1.52	138.90±1.84
Potassium	1017.40±23.74	1604.40±21.28
Sodium	97.00±0.50	20.10±0.14
Phosphorus	90.00±0.00	330.00±0.00
Iron	0.07±0.001	1.52±0.08
Zinc	0.22±0.04	1.02±0.06
Copper	0.03±0.004	0.11±0.01

Prince of Songkla University Pattani Campus

4.2 Optimization of culture conditions for *A. aceti* TISTR 102

4.2.1 Effect of different experimental culture substrates

The comparison among different culture media for their ability to enhance the viable cells of *A. aceti* TISTR 102 was investigated. The experimental media included GY as reference, coconut water and coconut water with banana juice supplemented at different volumes: 25, 50, 75 and 100% (v/v). Results are shown in Figure 6. Generally, *A. aceti* TISTR 102 grew well in most of the experimental media and attained at least 10^7 CFU/ml from 6 hrs onwards. Obviously, the early stationary phase was observed at 18-24 hrs within all treatments. The growth pattern of *A. aceti* TISTR 102 in the coconut water medium was comparable, even markedly better than the one in synthetic GY medium at the same 10% (w/v) total sugar content. The explanation conceivably was due to the abundance of nutrients and sufficient amount of sugar could entirely support the requirement for biomass production (Supakod and Wongwicharn, 2012). Approximately 2.61±0.13 g/100mL sugar content in coconut water insufficiently supports carbon source for microorganisms, results in sugar enhancement for their consumption. Thus, a good growth of *A. aceti* TISTR 102 in coconut water with additional sucrose was clearly observed.

In Figure 6a, during the first 18 hrs, the viable cells in all experiment media were insignificantly different. However, the media with presence of banana juice had statistically more cell viability than the coconut water as sole medium, especially during stationary phase. The increase in additional volume of banana juice from 25% to 100% (v/v) resulted in the enhancement of cell viability, up to more than 8.48 log CFU/mL. It might be explained by the presence of norepinephrine that was known as neurochemical substance. Norepinephrine was found in banana pulp (*Musa* x *paradisiaca*) with 325 ng/mL extract that predominantly had enhancement effects on the growth of several gram-negative bacterial species (Lyte, 1997). As mentioned by Kinney *et al.* (2000), norepinephrine belongs to catcholamines with the catechol-ring structure (i.e. siderophores or iron carriers) that enables to scavenge iron from the environment and to make the minerals in bacteria. Norepinephrine interaction was a biochemical metabolism that not all bacteria could perform and respond with positive effect (Belay, 2003), although gram-negative bacteria could respond better (Belay and Sonnenfeld, 2002). Thus, the enhancement effect of norepinephrine on *A. aceti* TISTR

102 growth has been doubt, resulted in nutritional factors in banana juice such as trace elements, minerals also might involve in the bacterial growth. The maximum growth pattern attained at 50% (v/v) added banana juice with approximately 8.82-9.07 log CFU/mL at stationary phase. The externally added banana juice up to 75% and 100% (v/v) showed the statistically insignificant growth pattern as compared to the pattern at 50% (v/v) added banana juice. Vega *et al*. (1988) reported that banana juice at 21% was the optimal concentration for culturing the lipid-accumulating yeast *Apiotrichum curvatum* ATCC 20509. Besides, they also pointed out the higher concentrations of banana juice even caused the adverse effect on the growth without explanation or hypothesis. In this experiment, in the treatment 50% (v/v) added banana juice, the juice was diluted with 6 folds from the original pulp that approximately similar concentration as previous research. In addition, the more banana juice added might form more precipitate, leading to prevent the oxygen transfer for bacterial uptake. Supakod and Wongwicharn (2012) found that the addition of enzyme-extracted banana juice into coconut water with ratio 1:1 (v/v) could enhance the cell viability, nearly 1.2 folds. However, this result showed that the same fold increase could be attained with banana juice volume reduced to half (i.e. 1 coconut water : 0.5 banna juice) and 0.221 hr^{-1} specific growth rate.

Different culture media had differently initial reducing sugar content, depending on the substrate and the volume of added banana juice (Figure 6b). The synthetic GY medium containing glucose as the sole carbon source had the highest reducing sugar with 21.56±0.85 g/100mL and sharply declined during the incubation time. Because of the high oxidative ability against monosaccharide, most of microorganism, including *Acetobacter* strains could consume glucose more effectively. Thus, the high sugar consumption in GY medium was observed the final sugar content was recorded with 14.24±0.22 g/100mL. The other media contained sucrose that obtained from their nutritional facts and externally supplemented, beside glucose. The initial reducing sugar contents were 6.48±0.69, 9.63±0.21, 11.50±0.16, 13.64±0.20 and 14.62±0.44 g/100mL in coconut water and coconut water with added banana juice at different volumes 25, 50, 75 and 100% (v/v), respectively. All these media had the similar trend in reducing sugar pattern that slightly decreased during the incubation time. De Ley and Schell (1959) asserted that *Acetobacter* strains could

Prince of Songkla University Pattani Campus

consume sucrose, and bacteria additionally even grew well in the experimental media (Figure 6a), might prove for sucrose consumption in this bacterial strain. It was possible that this bacterial strain predominantly consummed glucose to grow up, similarly in most of microorganisms. Bacteria increased their size and took time to metabolize the sucrose into simple sugar. The glucose obtained from sucrose metabolism could remain the reducing sugar contents, resulted in slightly changing during incubation time. The final reducing sugar contents were correspondingly 4.90±0.15, 6.60±0.11, 9.23±0.89, 9.60±0.60 and 11.21±0.64 g/100mL.

Bacteria consumed sugar and increased the population during incubation time, acetic acid was consequently produced as the biochemical product to make the pH values decrease. In all of the treatments, pH values reduced significantly in the first 24 hrs and changed insignificantly after that time (Figure 6c). The pH values of the synthetic GY medium with glucose as the main carbon source dramatically decreased and significantly different from the others, that previously confirmed by the consumption sugar pattern. The initial and the final pH values were recorded with 4.70±0.01 and 3.51±0.01, respectively. Because of acidic properties of banana juice with pH 4.44±0.02 (Table 5), the more volume of banana juice was added, the lower pH values were obtained. The initial pH values were 4.92±0.02, 4.85±0.02, 4.84±0.02, 4.82±0.02 and 4.85±0.02 in coconut water, and coconut water with added banana juice at different volumes 25, 50, 75 and 100% (v/v), respectively. pH values were statistically different among coconut water treatment and coconut water with 25 and 50% (w/v) added banana juice, but an insignificant difference was observed at 50% (v/v) added banana juice upwards. The final pH values corresponding were recorded with 3.93±0.01, 3.92±0.01, 3.69±0.01, 3.67±0.01 and 3.67±0.01.

In short, with the highest viable cells obtained during incubation time, coconut water standardized to 10% (w/v) sugar by sucrose with 50% added banana juice was the optimal treatment for the next experiments.

Prince of Songkla University Pattani Campus

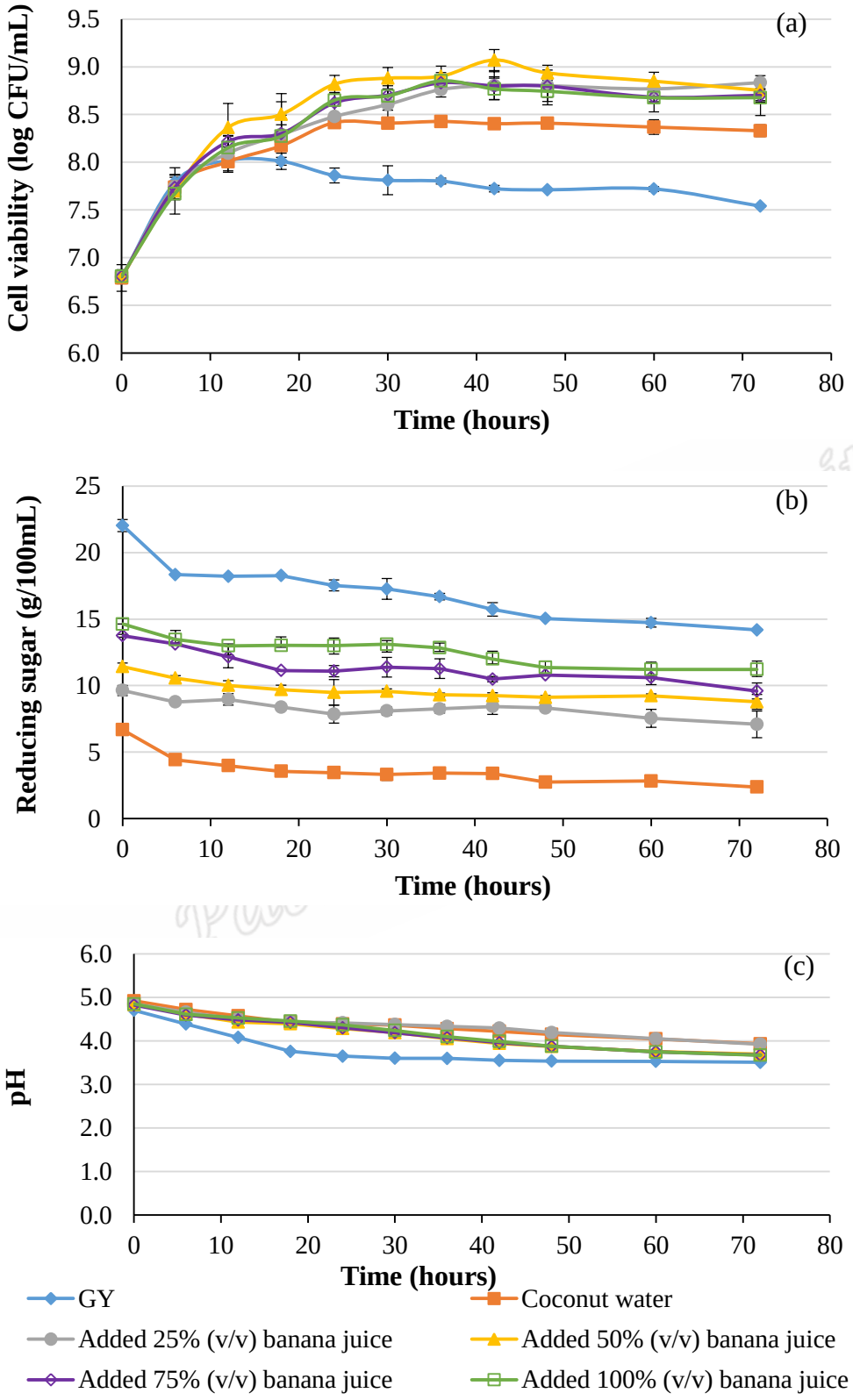

Figure 6 The growth of *A. aceti* TISTR 102 (a), reducing sugar content (b) and pH change (c) in different culture media

4.2.2 Effect of ammonium sulfate

The effects of amonium sufate with different concentrations of 0, 0.1, 0.3 and 0.5% (w/v) as nitrogen source on the growth of *A. aceti* TISTR 102, reducing sugar content and pH change are presented in Figure 7.

Ammonia is known as an important source of nutrients for bacteria because it contains nitrogen, which is used to make proteins and nucleic acids. Figure 7a pointed out that *A. aceti* TISTR 102 attained the early stationary phase after 18 hrs, did not grow remarkably in the presence of ammonium sulfate. There was an insignificant difference in cell viability at stationary phase within 0, 0.1 and 0.3% (w/v) concentrations: 8.68-8.77, 8.67-8.72 and 8.68-8.77 log CFU/mL. Correspondingly, the specific growth rates were 0.204, 0.202 and 0.200 hr^{-1}. Ammonium sulfate added up to 0.5% (w/v) showed relatively lower growth pattern with 8.62-8.68 log CFU/mL of viable cells and exponential growth rate 0.191 hr^{-1}. The physicochemical properties of coconut water and banana juice (Table 5) could explain for the results. Obviously, nitrogen content in coconut water was approximately 1.20±0.1 g/100 mL that was as same as the nitrogen content (i.e from 1% (w/v) yeast extract) in synthetic GY medium. The nitrogen content was in excess within the presence of banana juice (nitrogen 2.70±0.1 g/100 mL) and further supplemented ammonium sulfate. So far, there has not been exactly nitrogen content sufficient for this bacteria, but the nitrogen from ammonia salt varied among bacterial species and depended on culture medium. Wongsudaluck (2012) showed the effect of ammonium sulfate on growth of *A. aceti* TISTR 102 in palm sap juice medium and pointed out the higher bacterial growth patterns were obtained when ammonium sulfate was supplemented; but there was no significant difference in viable cells among the experimental concentrations 0.2, 0.4, 0.6 and 0.8% (w/v). The lower growth pattern obtained at 0.5% (w/v) ammonium sulfate concentration might be due to osmolarity of the medium (Müller *et al*., 2006).

The reducing sugar content decreased during the incubation time that confirmed the results of growth patterns (Figure 7b). Generally, the reducing sugar contents in all of the treatments dramatically decreased during the first 18 hours that corresponding to exponential phase of bacteria. During the later time, bacteria at stationary phase consumed sugar less than last phase did, resulted in the reducing sugar contents decreased slightly. There was an insignificant difference in reducing

sugar contents at the first 24 hrs; but at the 36th hr onwards, reducing sugar was continuously consumed more in control treatment as compared to those of counterparts. Approximately 6.60±0.15, 6.62±0.17; 6.58±0.12 g/100mL were initial reducing sugar contents; while 4.69±0.07, 5.20±0.02, 5.51±0.02 g/100 mL were the reducing sugar contents after 72 hrs incubation obtained at 0, 0.1 and 0.3% (w/v) ammonium sulfate. Additionally, the reducing sugar pattern at 0.5%(w/v) ammoinium sulfate treatment was relatively less as compared to the others with 6.63±0.15 and 5.75±0.07 g/100mL were initial and final reducing sugar contents, respectively. As mentioned above, the presence of ammonium sulfate could cause adverse effects due to either osmolarity. The synergic effects possibly occurred, especially at the later stationary phase with nutrients depletion and inhibitory products formation, which cause the decrease in bacterial population and sugar consumption rate.

The changes of pH values are presented in Fugure 7c. The more ammnium sulfate was added, the lower pH was obtained. As the results, the initial pH values were statistically different from one another: 4.89±0.01, 4.82±0.02, 4.77±0.02 and 4.74±0.02 for treatment control, 0.1, 0.3 and 0.5% (w/v) ammonium sulfate, respectively. However, these pH patterns changed in relatively similar trend. Indded, pH decreased dramatically in the first 24 hours and slightly changed in the later time. pH values in control treatment were significantly higher as compared to those of the counterparts. But, from the 36th hr onwards, when the sugar was continously consumed, pH values decreased more to be lower with the final pH 3.66±0.01. The pH values in treatment 0.1% ammonium sulfate decresed and just statistically different from the other treatments in the first 24 hours. Whereas, no significant difference in pH values was observed between data in treatments 0.3 and 0.5% (w/v) ammonium sulfate. The final pH values were recored with 3.75±0.02 and 3.74±0.00 and 3.75±0.01, correspondingly.

To sump up, from the obtained results, ammonium sulfate did not enhance the bacterial growth. So, the nitrogen source from ammonium sulfate supplementation could be reduced or cut out of the optimization process, leading to the cost reduction as culturing *A. aceti* TISTR 102

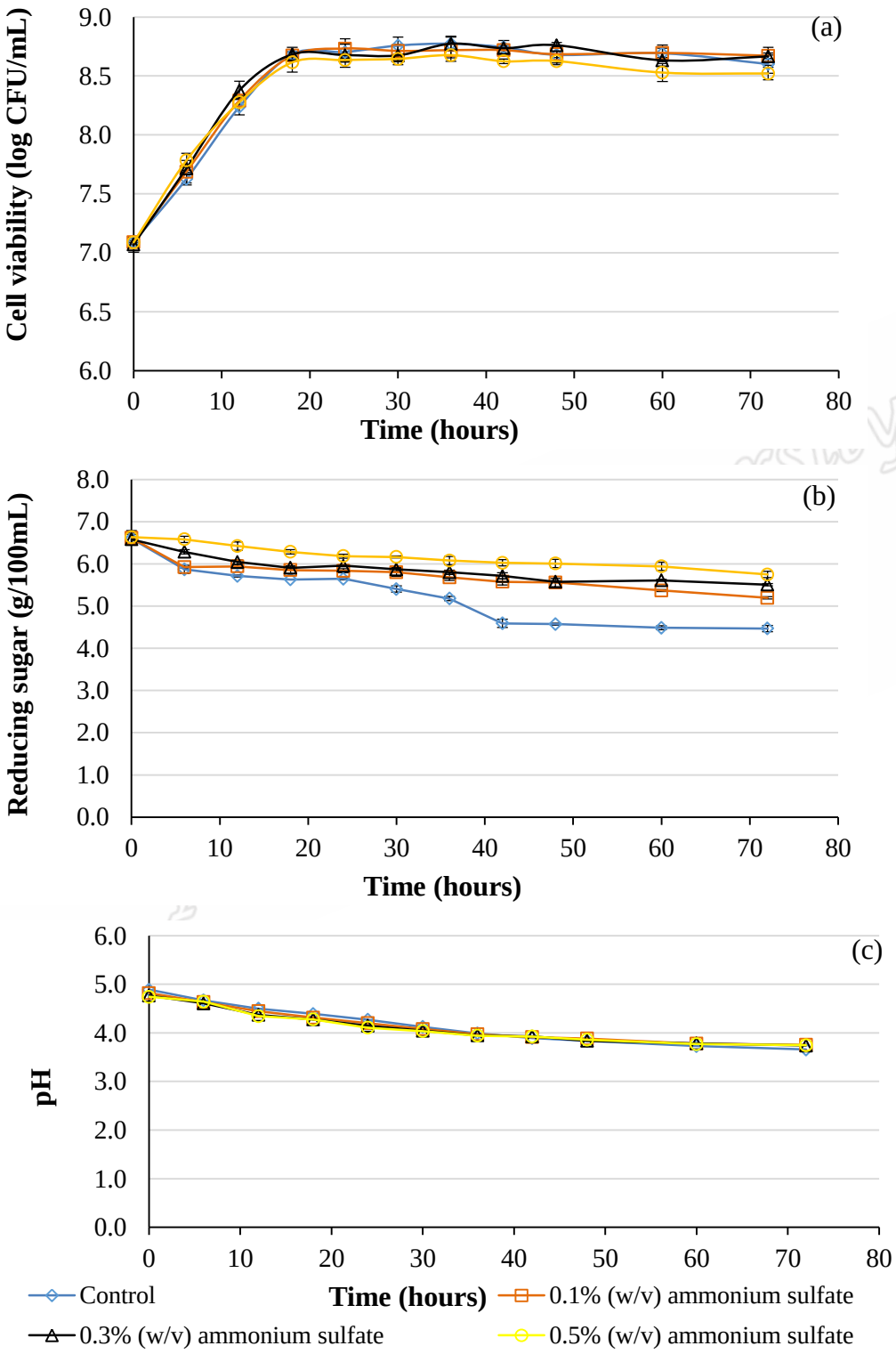

Figure 7 The growth of *A. aceti* TISTR 102 (a), reducing sugar content (b) and pH change (c) of coconut water with 50% (v/v) added banana juice medium at different concentrations of ammonium sulfate (w/v)

4.2.3 Effect of yeast extract

Yeast extract has been shown that apart from organic nitrogen source, it is an essential source of vitamin, trace elements, etc. The results of cell viability of *A. aceti* TISTR 102, reducing sugar content and pH change with various yeast extract concentrations of 0, 0.2, 0.6 and 1.0% (w.v) are presented in Figure 8.

Obviously, in Figure 8a, yeast extract does not not show enhancement effect on the bacterial growth through concentrations of 0, 0.2, 0.6 and 1.0% (w/v). Indeed, viable cells without yeast extract attained 8.37-8.50 log CFU/mL in stationary phase with the specific growth rate 0.144 hr^{-1}. Cell viability 8.36-8.43; 8.28-8.54 and 8.38-8.48 log CFU/mL were obtained at 0.2, 0.6 and 1.0% (w/v) yeast extract, respectively. Correspondingly, 0.150, 0.141 and 0.142 h^{-1} were the recorded specific growth rates. In several studies, yeast extract efficiently stimulated cell growth, even increased the production yield. For examples, Wongsudaluk (2012) studied effect of yeast extract on *A. aceti* TISTR 102 in palm juice at various concentrations 0.2, 0.4, 0.6 and 0.8% (w/v) and 0.4% (w/v) yeast extract concentration produced the highest cell viability. However, yeast extract just showed its positive effect on *Apiotrichum curvatum* ATCC 20509 biomass within the banana juice concentrations less than 19%; otherwise the enhancement effect was not observed at the higher 19% concentrations (Vega *et al.*, 1988). This study was in agreement with the experimental results to strongly confirm the sufficiently chemical requirements of banana juice for microbial growth enhancement. Thus, in case, yeast extract might be ignored, which is a beneficial result involves to economic issues.

Figure 8b presents the reducing sugar contents via the decreasing trend during incubation time. The initial reducing sugar contents were startistically different from one another with 7.90±0.07, 8.21±0.07, 8.35±0.07 and 8.45±0.05 g/100mL recorded at treatments of 0, 0.2, 0.6 and 1.0% (w/v) yeast extract, respectively. In spite of the difference in the initial contents, the reducing sugar patterns relatively tended to have similar trends. In most of treatments, the reducing sugar was remarkablly consumed in the first 24 hrs for exponential phase and insignificantly consumed in the later time for stationary phase. These results possibly confirmed the result of growth pattern above that did not have the significant difference in cell viability among these treatment. The final reducing sugar contents were recorded with 6.44±0.07,

6.58±0.07, 6.79±0.07 and 7.19±0.10 for treatment control, 0.2 0.6 and 1.0% (w/v) yeast extract, respectively.

The changes of pH values are performed in Figure 8c. The more yeast extract was added, the higher pH was obtained. Therefore, the initial pH value at control was 4.84±0.01 that statistically different from the others; while 4.86±0.01, 4.87±0.02 and 4.88±0.02 were the initial pH values in treatments 0.2, 0.6 and 1.0% (w/v) yeast extract, respecitively. Generally, in most of treatments, the pH changes remarkablly decreased in the first 24 hrs and insignificantly reduced in the later time. The pH values in control treatment were obviously lower than those in counterparts with the final pH value 3.82±0.01. For the other treatments, the pH values decreased toward similar trend but the higher pH values were recorded. Indeed, the final pH values were recored with 3.93±0.02 and 4.01±0.01 and 4.09±0.01 in treatments 0.2, 0.6 and 1.0% (w/v) yeast extract, respectively.

In short, yeast extract contained organic nitrogen source and essential source of vitamin, trace elements did not stimulate the bacterial growth within 0.2, 0.6 and 1.0% (w/v) concentrations. Therefore, yeast extract was possibly ignored in the culture medium composed from coconut water and added banana juice for economic benefits in micribial culturing

Prince of Songkla University Pattani Campus

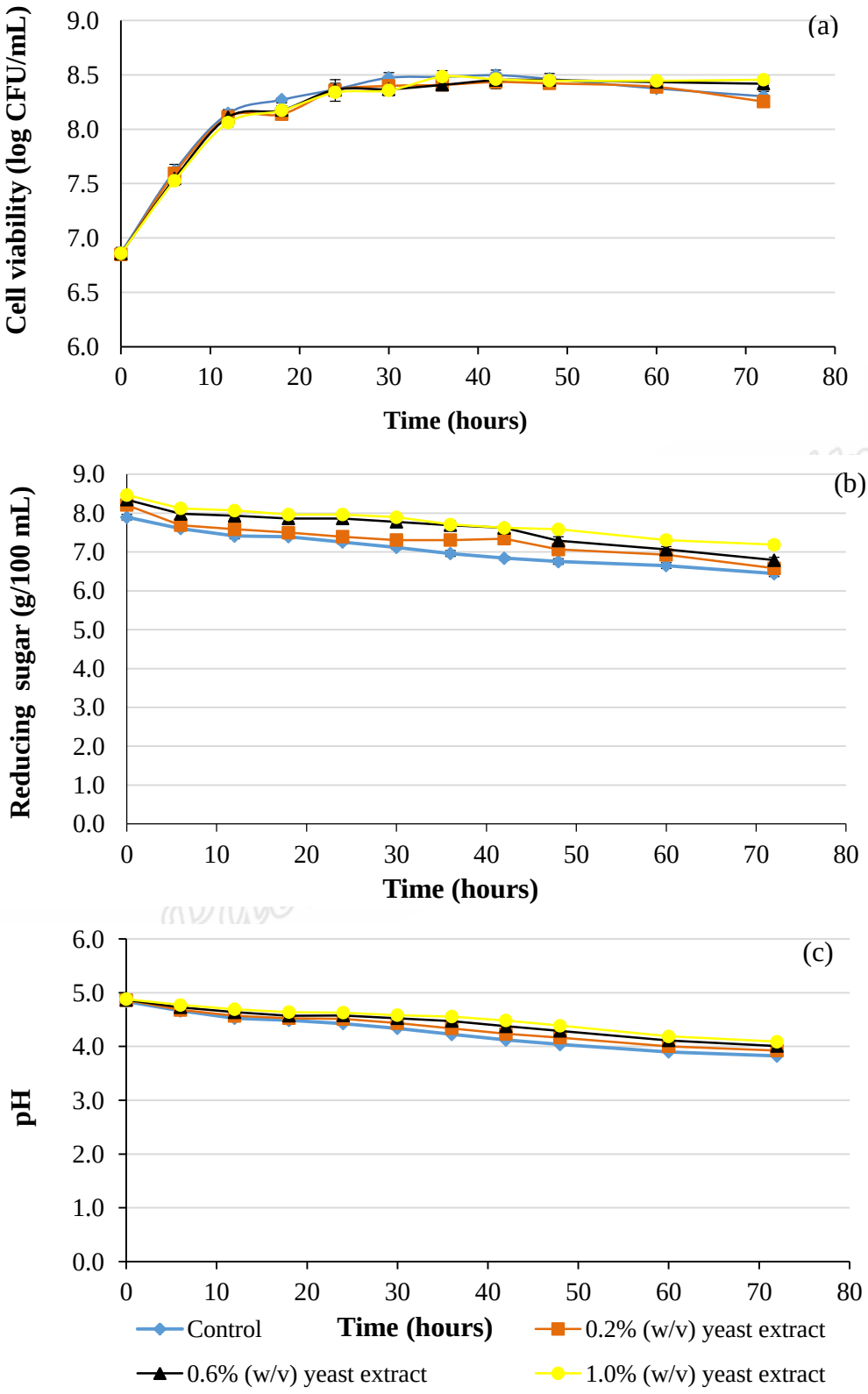

Figure 8 The growth of *A. aceti* TISTR 102 (a), reducing sugar content (b) and pH change (c) of coconut water with 50% (v/v) added banana juice medium at different concentartions of yeast extract (w/v)

4.2.4 Effect of shaking speed

Acetobacter aceti TISTR 102 cultured in coconut water with 50% (v/v) banana juice voulme without any external suplementation was incubated at two different shaking speeds 120 and 150 rpm for 72 hrs at 30^{o}C. The results are presented in Figure 9. Figure 9a points out that the shaking speed had a noticable effect on the viable cells: the higher speed was applied, the greater cell viablility was obtained. A range from 8.46-8.57 log CFU/mL and 8.56-8.67 log CFU/mL attained in the stationary phase at 120 and 150 rpm, respectively. Correspondingly, the specific growth rates were 0.167 h^{-1} and 0.177 h^{-1}. The effect of shaking speed was reported in research of Wongsudaluk (2012). She pointed out that *A. acei* TISTR 102 cultured in palm sap medium attained highest cell viability up to $7.8x10^{14}$ at 180 rpm , while $4.1x10^{10}$ and $2.3x10^{13}$ were recorded at 100 and 130 rpm, respectively after 4 days. Tantratia *et al*. (2005) found that *Acetobacter* TISTR 975 (*A. xylinum*) cultured in coconut water at 50 rpm showed the smallest number of cells. At the other higher shaking speeds (i.e.100 and 150 rpm), the same higher number of cells was observed. This could confirm for dissolved oxygen increasing the growth of the cells and the excess oxygen in the medium did not further increase cell production after cell number optimization. *Acetobacter* genus, especially *A. aceti* TISTR 102 belongs to the aerobic microorganisms whose rate of growth and metabolism depend upon the amount of dissolved oxygen available. But, oxygen demonstrates the dissolving limitation, so a compulsory liquid aeration might be necessary to provide dissolved oxygen. According to Tesfeya *et al*. (2000), shaking broke the large bubbles formed at the entrance of air by bacterial clumps or biofilm formation, then oxygen transfered from the gas phase to the liquid phase increased. In addition, shaking obviously helps the medium to be almost equally distributed and prevents the bacteria sediment in the bottle of the flask. Moreover, the presence of turbid suspension from insoluble solid substances in banana pulp inhibited the microbial growth was a significant issue. The shaking, in this case, possibly prevented the precipitate formation and improved oxygen uptake.

The reduction in reducing sugar contents is presented in Figure 9b. Obviously, the reducing sugar contents decreased during the incubation time, and the higher consumption rate attained at the higher shaking speed. The difference in reducing

sugar content between two treatments was significant in the first 30 hours that corresponded to exponential and early stationary phases. In the later time, the reducing sugar contents in two treatments were not statistically different. The initial reducing sugar contents were 7.57±0.15 and 7.53±0.05, while 6.24±0.07 and 6.04±0.05 were the final reducing sugar contents in treatments 120 and 150 rpm, respectively.

The pH changes showed in Figure 9c were in contrast to results of cell viability and sugar consumption. The pH values were not statistically different between two treatments, which might infer that shaking speed might not enhance the acetic acid production yield. Indeed, 4.91±0.01 and 4.92±0.00 were the initial pH values without statistical difference; the final pH values were recorded with 3.80±0.01 and 3.83±0.01 for treatments 120 and 150 rpm, respectively.

Prince of Songkla University Pattani Campus

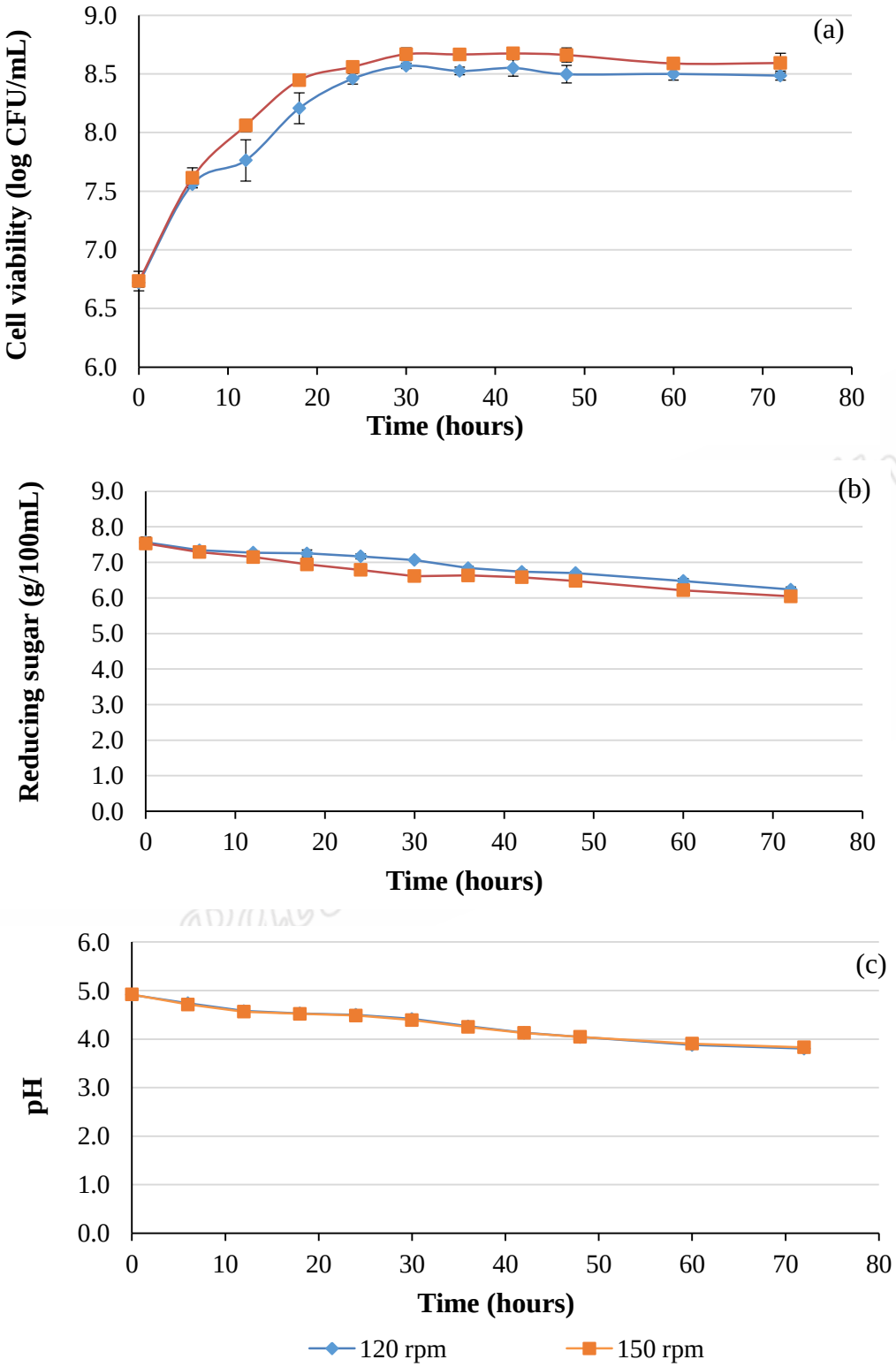

Figure 9 The growth of *A. aceti* TISTR 102, reducing sugar content (b) and pH change (c) in coconut water with 50% (v/v) added banana juice at different shaking speeds

4.3 Preparation and storage of *A. aceti* TISTR 102 starter powder

4.3.1 Effect of sucrose concentrations on bacterial cells in drying.

The comparison of protective effects on survival rate and cell viability of *A. aceti* TISTR 102 at different sucrose concentrations in convective-air drying are presented in Figure 10 and Figure 11.

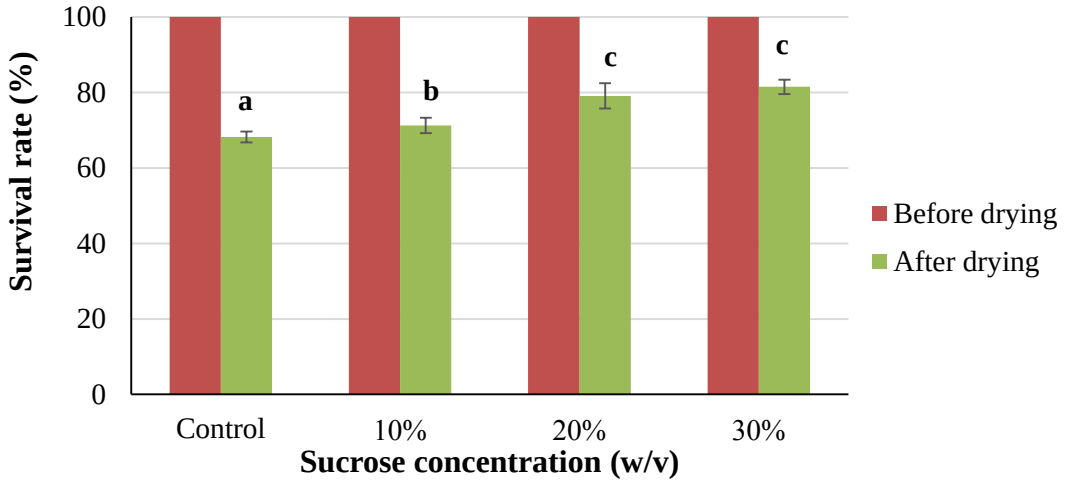

Figure 10 The survival rate (%) of *A. aceti* TISTR 102 starter powder at different sucrose concentrations before and after drying

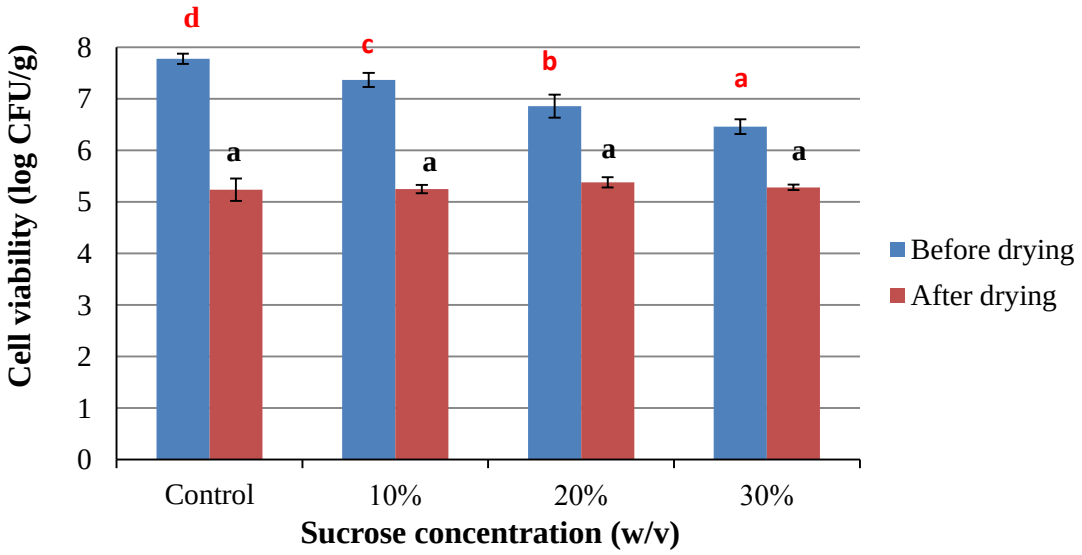

Figure 11 The cell viability of *A. aceti* TISTR 102 starter powder at different sucrose concentrations before and after drying

Generally, sucrose had significantly protective effect on the viable cells of *Acetobacter aceti* TISTR 102 starter powder ($p \leq 0.05$). The pellet suspended in sucrose solutions could recover with higher survival rates as compared to the one suspended in distilled water (control treatment). In Figure 10, the increasing of sucrose concentration from 0 to 20% (w/v) increased the recovered cell viability after drying, while the higher concentration at 30% (w/v) sucrose did not show significantly protective effect anymore. Indeed, a significant difference in survival rate was observed among treatments of 0,10 and 20% (w/v) sucrose with 68.24±1.42, 71.24±2.04 and 79.07±3.35(%), respectively ($p \leq 0.05$). The survival rate 81.50±1.91(%) that was insignificantly different was recorded at 30% (w/v) sucrose. Additionally, the initial cell viability of the starter powder decreased when the sucrose concentration increased (Figure 11). The initial viable cells in starter powder were statistically different from one another with 7.78±0.10, 7.37±0.14, 6.86±0.23 and 6.46±0.14 log CFU/g at treatment 0, 10, 20 and 30% (w/v) sucrose, respectively. However, after drying, the viable were correspondingly 5.24±0.22, 5.25±0.08, 5.38±0.10 and 5.28±0.05 log CFU/g without any significant difference. In fact, sucrose solution possibly induced the osmotic shock for microorganisms when they were re-suspended into. At the high sucrose concentrations, the decrease in viable cells might occur because of the primary shock responses, resulted in the lower initial viable cells were obtained at the higher sucrose concentrations. However, the osmotic shock would be considered as pre-adaption process, from which the cells were survived, and then further were protected by the accumulation of compatible solutes during drying process. Several studies on protective effect of sucrose were reported, especially in freeze-drying process. Leslie *et al*. (1995) reported that without treating in sugar solution *E. coli* and *B. thuringiensis* survived only 8% and 14%, respectively. But, the survival rates after freeze drying increased up to 56% and 44%, respectively with the presence of 100nM sucrose solution. Nomura *et al.* (1998) showed that *Acetobacter aceti* IFO 3284 cells that were lyophilized with 30% (w/v) sucrose could remain cell viability higher than 1 log cycle during 7-week storage with only 20% aldehyde oxidase (i.e. bacterial enzyme activity) decrease as compared with non-protected cells. Palmfeldt *et al*. (2003) found out that the protective effect of sucrose concentrations on the antifungal bacterium *Pseudomonas chlororaphis* during freeze-

Prince of Songkla University Pattani Campus

drying process was dependent on sucrose concentrations. They reported that the highest survival was obtained with sucrose concentrations in the range 50-130 g/l (i.e. corresponding to 146-380 mM). Otherwise, the negative effect was observed at the concentration up to 300g/L (i.e. 876 mM). So far, the protective effects of sugars, especially sucrose, were performed almost in freeze-drying process, while there has been lack of study on its effect in mild-temperature drying. The possibly protective hypotheses against to thermal injury were reviewed by Santivarangkna *et al*. (2008). They stated that the phospholipid membrane tended to change from the liquid crystalline to gel phase as water removed during drying process. There was a critical point at which the lipids were still fluid and molten, but they began changing to gel phase and the temperature at this point was called membrane phase transition temperature (T_m). The lipids crystallization was dependent on transition temperature: the lipids had higher transition temperature tended to crystalize first. The crystallization would not occur simultaneously for all classes of lipids because of the difference in structure, components, transition temperature, etc, resulted in the phase separation and consequent leakage of membrane. The presence of sugar could depress the membrane phase transition temperature to circumvent the gel phase transition and remain the liquid phase of the cell membrane. The mechanisms were proposed that the water molecules around polar head groups were replaced by the macromolecules substances (e.g. sugars) to form the high-affinity bond for membrane stabilization. Concretely, the hydrogen bonds were formed due to the interaction between hydroxyl group of sugars and the phosphate groups at the surface of the bilayer. Additionally, for another mechanism, sugars, especially sucrose could interact with protein by hydrogen bonds to prevent protein from denaturation in drying (Oldenhof *et al*., 2005; Morgan *et al*., 2006)

The moisture content before and after drying are presented in Table 6. There was an inverse proportion between sucrose concentration and moisture content of the starter powder before druing. Indeed, moisture contents 32.86±0.37, 30.79±0.57, 29.70±0.35 and 28.59±0.27 (% wb) were recorded at treatments 0, 10, 20 and 30% (w/v) sucrose, respectively. The reason was posible due to the relationship in solute and solvent: the higher sucrose concentrations intended to contain less unbound water, resulted in the lower initial moisture contents for starter powtder and adversely.

Prince of Songkla University Pattani Campus

However, after 12 hours drying, there was no a significant difference in final moisture content among sucorse-suspending treatments: 8.85±0.22, 9.18±0.41 and 9.30±0.31 (% wb) were recorded for treatment 10, 20 and 30% (w/v) sucrose, respectively. The significantly lower moisture content at 8.27±0.08 (% wb) for the control treatment was possibly due to the more free water in solvent that spontaneously evaporated undergo heat effect.

The results of water activity are also shown in Table 6. Similar trend was found in initial water activity with a significant difference in initial water activity. The more moisture content was contained, the higher water activity was recorded. Indeed, the water activity of starter powder before drying were 0.9465±0.001, 0.9352±0.002, 0.9264±0.001 and 0.9170±0.002 at treatment control, 10, 20 and 30% (w/v) sucrose, respectively. The final water activity coresspondingly were recorded with 0.5517±0.019, 0.5626±0.017, 0.5428±0.011 and 0.5451±0.004 without significant difference among treatments.

Table 6 Moisture content and water activity of *A. aceti* TISTR 102 starter powder suspended at different sucrose concentrations before and after drying

	Treatments	**Before drying**	**After drying**
Moisture content	Control	32.86±0.37 ^{d}	8.27±0.08 ^{b}
	10% (w/v) sucrose	30.79±0.57 ^{c}	8.85±0.22 ^{a}
	20% (w/v) sucrose	29.70±0.35 ^{b}	9.18±0.41 ^{a}
	30% (w/v) sucrose	28.59±0.27 ^{a}	9.30±0.31 ^{a}
Water activity	Control	0.947±0.001 ^{d}	0.552±0.019 ^{a}
	10% (w/v) sucrose	0.935±0.002 ^{c}	0.563±0.017 ^{a}
	20% (w/v) sucrose	0.926±0.001 ^{b}	0.543±0.011 ^{a}
	30% (w/v) sucrose	0.917±0.002 ^{a}	0.545±0.004 ^{a}

Data were represented as mean±SD from 3 replications. Mean values each column followed by the different superscript letter were significantly different at $p \leq 0.05$

Prince of Songkla University Pattani Campus

4.3.2 Effects of silica gel on shelf life of starter powder during storage

The results of cell viability, moisture content and water activity of *A. aceti* TISTR 102 starter powder during storage at 4°C without (control) and with silica gel are presented in Figure 12 and Figure 13, respectively.

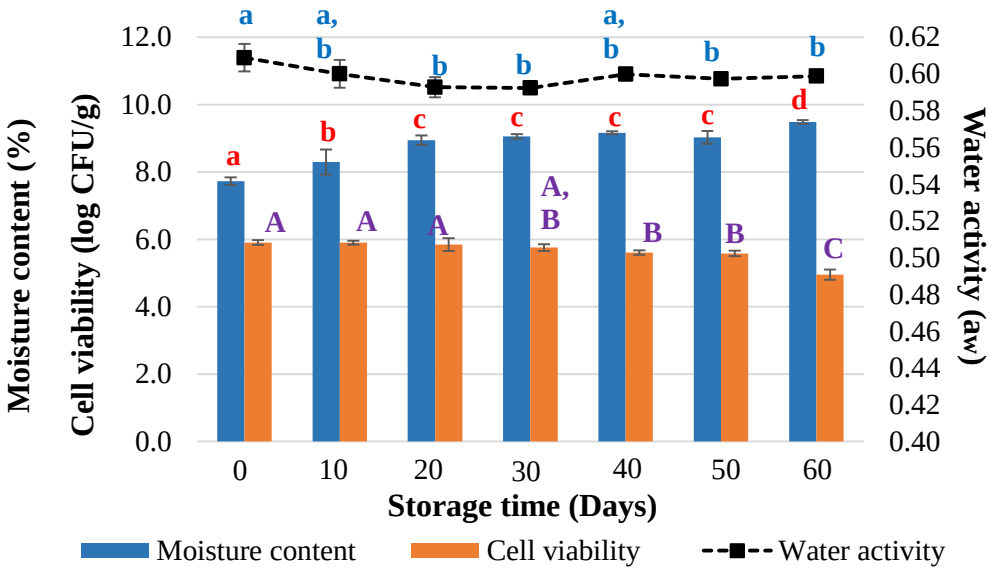

Figure 12 Moisture content, cell viability and water activity of *A .aceti* TISTR 102 starter powder during storage time without silica gel

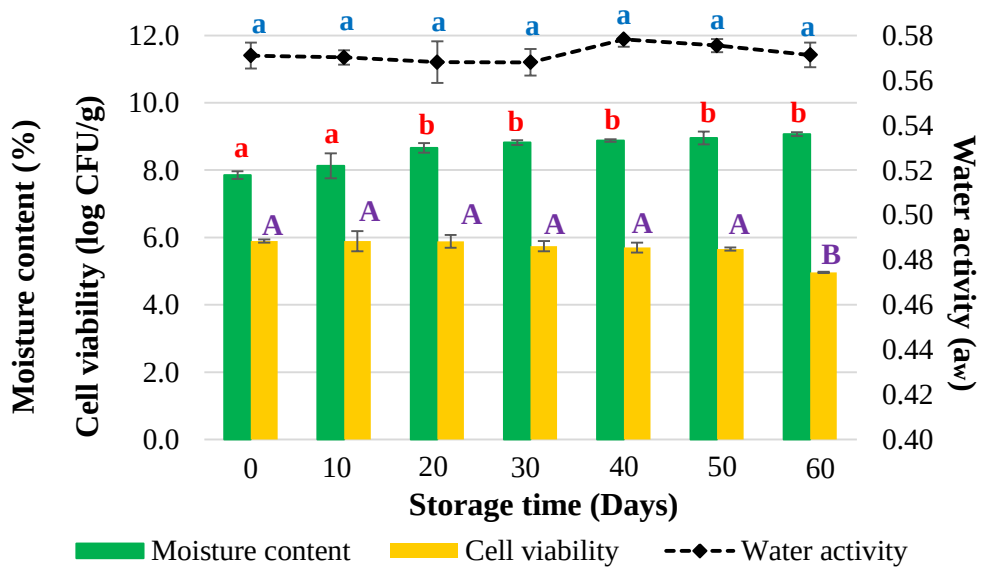

Figure 13 Moisture content, cell viability and water activity of *A. aceti* TISTR 102 starter powder during storage time with silica gel

Generally, silica gel had significant effect on the moisture content and cell viability of *A. aceti* TISTR 102 starter powder during storage, while water activity did not significantly change. In Figure 12, without silica gel, the starter powder had initial viable cells 5.91±0.07 log CFU/g that remained within 30 days. The viable cells statistically decreased to 5.76±0.10 log CFU/g after 30 days, and then maintained during next 20 days before sharply dropped to 4.95±0.16 log CFU/g at the last day of storage period. In contrast, with the presence of silica gel (Figure 13), there was no statistical difference in results of cell viability within 50 days storage. The initial viable cells and the viable cells of starter powder after 50 days were 5.89±0.05 log CFU/g and 5.65±0.05 log CFU/g, respectively. After 50 days, the viable cells significantly decreased and dropped to 4.96±0.02 log CFU/g at the last day.

The increasing in storage time increased the moisture content of starter powder. Without silica gel, the moisture content was initially 7.73±0.11 and increased remarkably to 8.30±0.37 and 8.94±0.14 (% wb) after 10 and 20 days storage, respectively. The moisture content then insignificantly changed within 1 month later before significantly increased to 9.49±0.06 (% wb) at the last day of storage period. In the other hand, silica gel showed its effect when the initial moisture content of starter powder was 7.85±0.32 (% wb) that slowly increased to 8.13±0.31 (% wb) after 20 days. The moisture content then statistically remained during storage time later.

The silica gel was packed with the aluminum foil pouch of starter powder in PE bag as an adsorbent to maintain the relative humidity or water activity of surrounding area. In this research, silica gel efficiently maintained the water activity of starter powder when no statistical change in results was observed up to 60 days. The water activity was insignificantly increased in storage period with a range from 0.5710±0.006 to 0.5782±0.003. Otherwise, the starter powder stored without silica gel had the slight changes in water activity during 2 month storage.

So far, there have been not many studies on desiccant's activity (e.g. silica gel) in storage the starter powder. Dianawati *et al*. (2013) compared the cell viability of freeze-dried and spray-dried microencapsulated *Lactobacillus acidophilus* and *Lactococcus lactis* ssp. *cremoris* during storage at room temperature with silica gel and other desiccants (e.g. NaOH and LiCl). Although silica gel worked as not well as the counterparts did; it still had significant protective effect on cell viability of both

Prince of Songkla University Pattani Campus

spray-dried microencapsulated cultures during 10 week storage compared to the control without desiccants. Similar trend for cell viability was observed in this research when *A. aceti* TISTR 102 starter powder could maintain its viable cells almost 2 months at 4^{o}C in vacuum-aluminum foil sealed pouch with presence of silica gel. According to Fu and Chen (2011), the decrease of cell viability involved to free-movement of molecules (e.g. water, oxygen) and consequent biochemical reactions. Obviously, vacuum-sealed aluminum foil pouch (i.e without oxygen), favorable storage temperature 4^{o}C and silica gel for controlling relative humidity possibly could prevent or slow down some detrimental reactions for survival of dried cell.

Prince of Songkla University
Pattani Campus

4.4 Preparation of vinegar from coconut water using baker's yeast and *A. aceti* TISTR 102 starter powder

4.4.1 Alcoholic fermentation: Effect of baker's yeast concentration

Coconut water at 14% (w/v) sugar content was fermented into ethanol with different baker's yeast concentrations of 0.2, 0.4 and 0.6% (w/v) at 30±2^{o}C in 7 days at static condition. The result of ethanol production is shown in Figure 14a. Generally, the maximal ethanol content attained approximately 9% (v/v) for all of the treatments, which means that the baker's yeast concentration did not affect the ethanol content. However, it was a significant factor on fermentation process performance. The higher baker's yeast concentration was inoculated; the produced ethanol contents attained in the shorter time, resulted in the higher fermentation rate. Indeed, baker's yeast at 0.2% (w/v) produced ethanol content that linearly increased within 3 days before stagnating during later time. Correspondingly, the ethanol contents were recorded with 3.64±0.08, 7.76±0.20 and 9.92±0.18 (% v/v). Likewise, the ethanol content increased sharply to 6.18±0.22 and 9.81±0.18 (%) within 2 days and statistically remained during later when coconut water was fermented at 0.4% (w/v) baker's yeast. Similar trend was found in with 0.6% (w/v) baker's yeast concentration, but the significantly higher ethanol contents were 7.57±0.24 and 9.86±0.10 (%), correspondingly.

The increase in ethanol content decreased the total sugar content during the fermentation time. The result of total sugar is shown in Figure 14b. Obviously, the higher baker's yeast concentration was inoculated, the faster total sugar was consumed. At 0.6% (w/v) baker's yeast, the total sugar content sharply declined to 2.70±0.15 and 0.07±0.02 (g/100mL) within 2 days that then statistically stagnated. Also, a slightly slower decreasing trend was observed at 0.4% (w/v) baker's yeast with 6.19±0.58 and 0.13±0.01 (g/100mL) total sugar contents were recorded after 2 fermentation days before being statistically constant in the further time. Otherwise, baker's yeast at 0.2% (w/v) took almost 3 days to consume sugar completely at 0.34±0.04 g/100mL.

The total soluble solid intended to decrease with similar trend in sugar consumption profile and the results are presented in Figure 14c. With 0.2% (w/v) baker's yeast, coconut water initially was at 18.0±0.2^{o}Brix total soluble solid that

Prince of Songkla University Pattani Campus

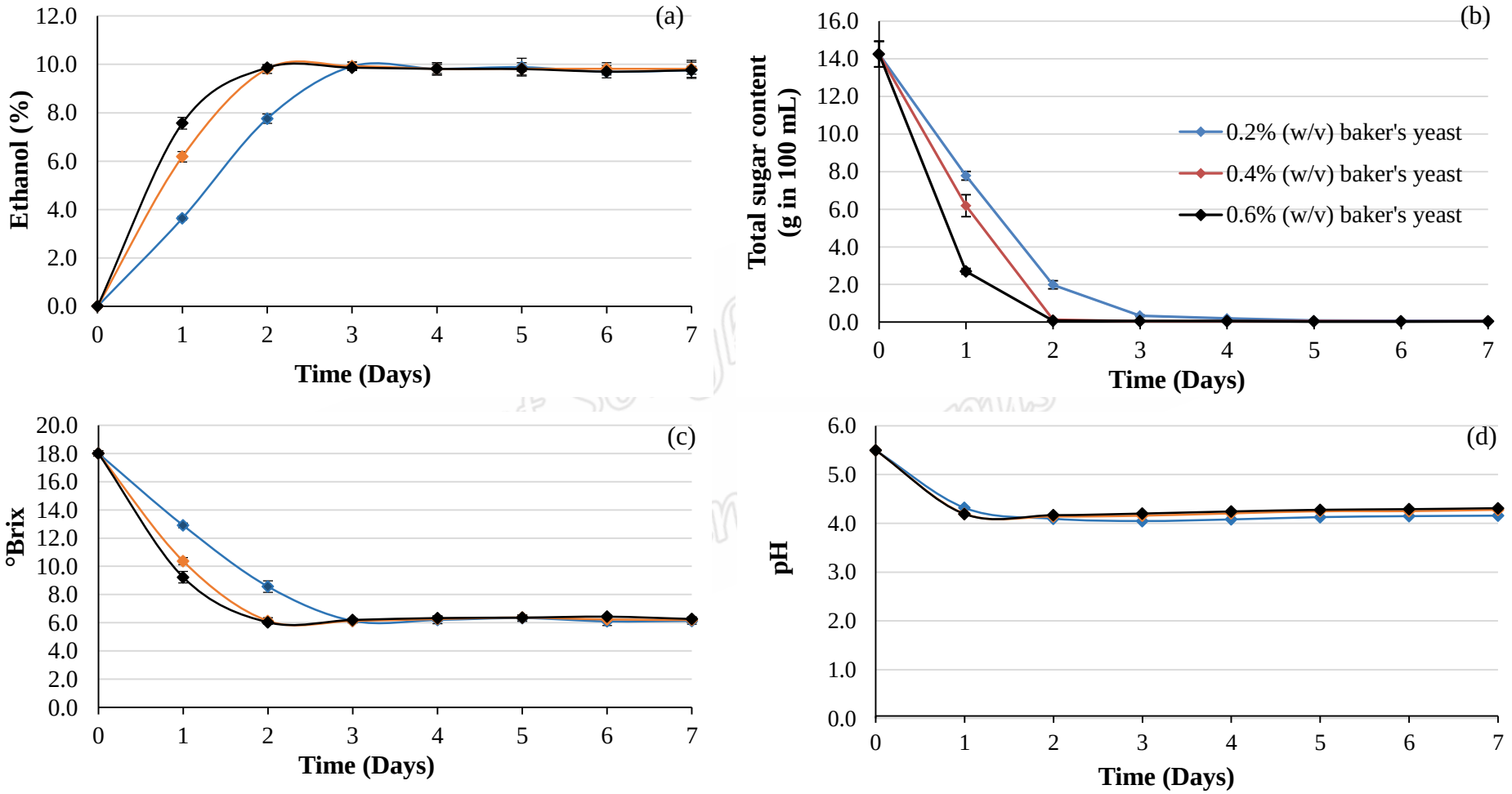

Figure 14 The ethanol content (a), total sugar content (b), total soluble solid (c) and pH change (d) of coconut water fermented with different baker's yeast concentrations

59

declined sharply to 12.9±0.2, 8.6±0.4 and 6.2±0.1 (^{o}Brix) within the first three days before being constant during the further time. A linear decrease otherwise was observed within only 2 days when coconut water was fermented with 0.4 and 0.6% (w/v) baker's yeast. Correspondingly, total soluble solid at 10.4±0.3 and 9.2±0.4 (^{o}Brix) were recorded after 1 day and finally attained 6.0±0.1^{o}Brix without any changes later on.

Figure 14d shows the pH changes during fermentation time with different baker's yeast concentrations. There was no significant difference in pH values among treatments, exception for the later time when the fermentation almost finished. In most of cases, the pH values dropped sharply after 1 day of fermentation, gradually decreased and then slightly increased again after the fermentation complete. The final pH values were recorded 4.16±0.01, 4.28±0.03 and 4.31±0.02 at 0.2%, 0.4% and 0.6% (w/v) baker's yeast, respectively.

So far, the potentiality of using baker's yeast in alcoholic fermentation has been investigated in several studies, in which the yeast concentration was one of the significant factors on fermentation process performance. In jackfruit fermentation, the increase in baker's yeast concentration from 0.5 to 1.0% (w/v) obviously increased the fermentation rate, resulted in shortening the fermentation time within 9 days and 7 days, respectively (Kumoro *et al*., 2012). The increase in baker's yeast concentration from 1.0% (w/v) upwards affected on fermentation performance without any significant difference (Akin-Osanaiye, 2008, Kumoro *et al*., 2012). Moreover, the yeast concentrations more than 30.0 g/L or 3.0% (w/v) also could cause the minor inhibition in the ethanol yield (Matloob, 2014). The explanation possibly due to the high propagation of yeast cells in fermentable substrate and their competition in nutrient consumption (Ndip *et al*., 2001). Concretely, the yeast inoculated into the fermenting liquid with proper conditions (e.g. temperature, nutrient, pH, etc) easily increased the number of cells during fermentation process. The more yeast cells inoculated the higher number of cells produced, resulted in the high competition among yeast cells in nutrients consumption. Additionally, other reason was due to the high ethanol content in the beginning of fermentation, which usually performed with the rapid fermentation rate possibly caused negative effect on further growth of the yeast cells, resulted in the decrease in ethanol yield (Kumoro *et al*., 2012). The

Prince of Songkla University Pattani Campus

experimental results confirmed the previous studies when baker's yeast concentration in a range from 0.2 to 0.6% (w/v) had a positive effect on the alcoholic fermentation performance, especially fermentation time. The higher baker's yeast concentration was inoculated, the more ethanol contents were produced in the shorter time, resulted in the higher fermentation rate. The fermentation rate was calculated by the quotient between the produced ethanol and the fermentation time required to reach that concentration. Therefore, the ethanol production rates were 3.31, 4.91 and 4.93 (% per day) when the baker's yeast inoculated at 0.2, 0.4 and 0.6% (w/v), respectively.

From this experiment, the baker's yeast at 0.4% (w/v) was adequate to produce ethanol within proper time as compared to other treatments. Therefore, 0.4% (w/v) baker's yeast was chosen for the next experiments.

4.4.2 Alcoholic fermentation: Effect of sugar concentration

Coconut water at 12, 16 and 20% (w/v) sugar content was fermented with 0.4% (w/v) baker's yeast at 30±2^{o}C in 7 days at static condition. The results of ethanol production, total sugar content, total soluble solid reduction and pH change are presented in Figure 15.

Figure 15a clearly shows the dependence of produced ethanol contents on difference initial sugar contents: there was a proportional relationship between the sugar content and the ethanol production. Indeed, the higher substrate concentrations may achieve higher ethanol production, but a longer incubation time was required. At 12% (w/v) sugar content, the thanol content linearly increased to 6.27±0.14% and fermentation was completed within 1 day as the ethanol content statistically constant during the later time. The coconut water at 16% (w/v) sugar content produced ethanol content up to 9.61±0.17% (w/v) within 2 days before stagnating in the further time. Otherwise, the increase in ethanol production prolonged within 3 days to attain 12.37±0.06% ethanol content with coconut water at 20% (w/v) sugar content. The fermentaion rates 6.27, 4.80 and 4.12 (% per day) were calculated when coconut water at 12, 16 and 20% (w/v) the sugar content fermented, respectively.

The increase in ethanol content decreased the total sugar content during the fermentation time. The result of total sugar pattern is shown in Figure 15b. Obviously, the higher sugar content took longer time to be consumed completely by baker's

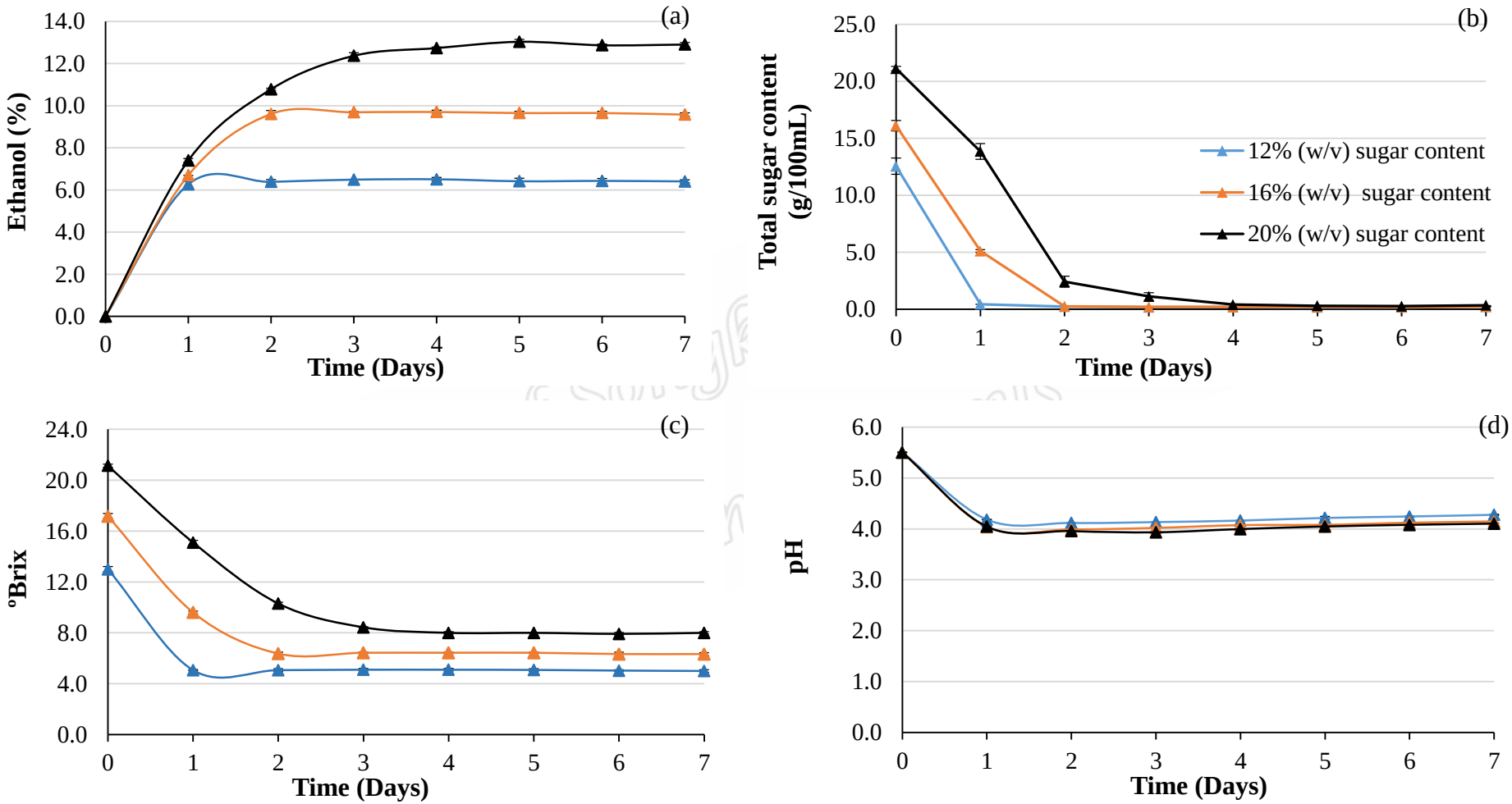

Figure 15 The ethanol content (a), total sugar content (b), total soluble solid (c) and pH change (d) of coconut water fermented at different sugar contents

yeast, and the increase in the initial sugar content also increased the residual sugar content. Coconut water contained 12.55±0.71 g/100mL total sugar content that was completely consumed within 1 day and remained 0.44±0.00 g/100mL without any more consumption thereafter. Otherwise, at 16% (w/v) sugar content, baker's yeast took longer time to consume almost the sugar and metabolized into ethanol. Indeed, the initially total sugar content was 16.11±0.46 g/100 mL that linearly decreased to 5.11±0.11 and sharply dropped to 0.25±0.01g/100mL within 2 days. At 20% (w/v) sugar content, a linear decline from 21.12±0.19 g/100mL total sugar content to 2.41±0.49 g/100mL was also observed in the first two days. There was a slight decrease in the next day to 1.12±0.34 g/100 mL before almost completing fermentation at 0.41±0.02 g/100 mL.

The total soluble solid similarly tended to decrease during fermentation time (Figure 15c). Generally, the increase in sugar content from 12, 16 and 20 % (w/v) increased the total soluble solid of the fermenting substrate (i.e. coconut water) at 13.0±0.20, 17.17±0.21 and 21.13±0.12 ^{o}Brix, respectively. Correspondingly, the final total soluble solid values remained constant at 5.10±0.10, 6.37±0.12 and 8.00±0.10 ^{o}Brix and fixed within 1, 2 and 3 days fermentation.

The pH value dropped significantly from 5.50±0.02 in the first day and the lower pH values were obtained with the increase in substrate concentration from 12, 16 and 20% (w/v) sugar. The pH values decreased to 4.04±0.01, 4.05±0.01 and 4.9±0.01 for treatments of 12, 16 and 20% (w/v) sugar before slightly increasing again when the fermentation had almost completed (Figure 15d). The final pH values were recorded 4.28±0.01, 4.15±0.01 and 4.10±0.01 at treatments of 12, 16 and 20% (w/v) sugar, respectively.

For alcoholic fermentation, substrate concentration (i.e. sugar content) is an important factor in fermentation. The experimental results strongly agreed to several previous researches: the higher substrate concentration fermented, the higher ethanol content within the longer time fermentation completely performed (Asli, 2010, Kumoro *et al*., 2012). In this experiment, the ethanol production rate were calculated and recorded 6.27, 4.80 % per day with the initial sugar contents of 12% to 16%; respectively. Otherwise, the lower ethanol production rate with 4.12 % per day was obtained at 20% sugar content. Besides, at the substrate concentrations up to 25.0 g/L,

Prince of Songkla University Pattani Campus

the ethanol production could be decreased or inhibited (Charoenchai *et al*., 1998, Grahovac *et al*., 2012). Nagodawithana *et al*. (1974) reported that the high substrate concentration might cause the osmotic shock that affected directly on the rate of glycolysis - an important biochemical metabolism to support energy for the survival of yeast cells. In addition, when the high ethanol concentrations formed and by-products accumulated, resulted in the pH change to cause inhibition (Lin *et al*., 2012).

In short, from this experiment, the treatment of 12% (w/v) sugar content could produce approximately 6% (v/v) ethanol content within proper time. Therefore, coconut water at 12% (w/v) sugar content was chosen for the next experiments.

4.4.3 Acetification: Effect of *A. aceti* TISTR 102 starter powder

Fermentation coconut water at 12% (w/v) sugar content with 0.4% (w/v) baker's yeast performed in 24 hours to obtain approximately 6% (w/v) ethanol concentration. Then, the *A.aceti* TISTR 102 starter powder was consequently inoculated into alcoholic substrate at different concentrations of 0.5, 1.0, 1.5 and 2.0% (w/v) for acetificcation at 30±2^{o}C within 30 days at static condition. The starter powder contained 1.26x10^{7} CFU/g with 7.17±0.17 (%) moisture content and 0.41±0.18 water activity.

The result of ethanol content is shown in Figure 16a. Generally, after inoculating starter powder, the ethanol content remained almost 3 days before sharply decreasing in later on. In all of the treatments, the ethanol content intended to decline linearly that started from the 6th day onwards until the ethanol was run out. The treatment of 0.5% (w/v) starter powder showed the slightly higher consumption rate, which could be inferred from the significantly lower residual ethanol content at any sampling time. There was no statistical difference in residual ethanol content was obsevred among the treatments 1.0, 1.5 and 2.0% (w/v) starter powder. Obviously, the ethanol was consumed completely after 18 days with treatment of 0.5% (w/v) starter powder, while 0.29±0.05, 0.31±0.18 and 0.37±0.23 (%) was recorded at treatments of 1.0, 1.5 and 2.0% (w/v) starter powder at the same time.

The decrease in ethanol content increased the acetic acid content during the fermentation time. The result of acidity production is presented in Figure 16b. The acetic acid started to be produced after 3 days from inoculating and also increased linearly before going to be stationary. Obviously, the treatment of 0.5% (w/v) starter

Prince of Songkla University Pattani Campus

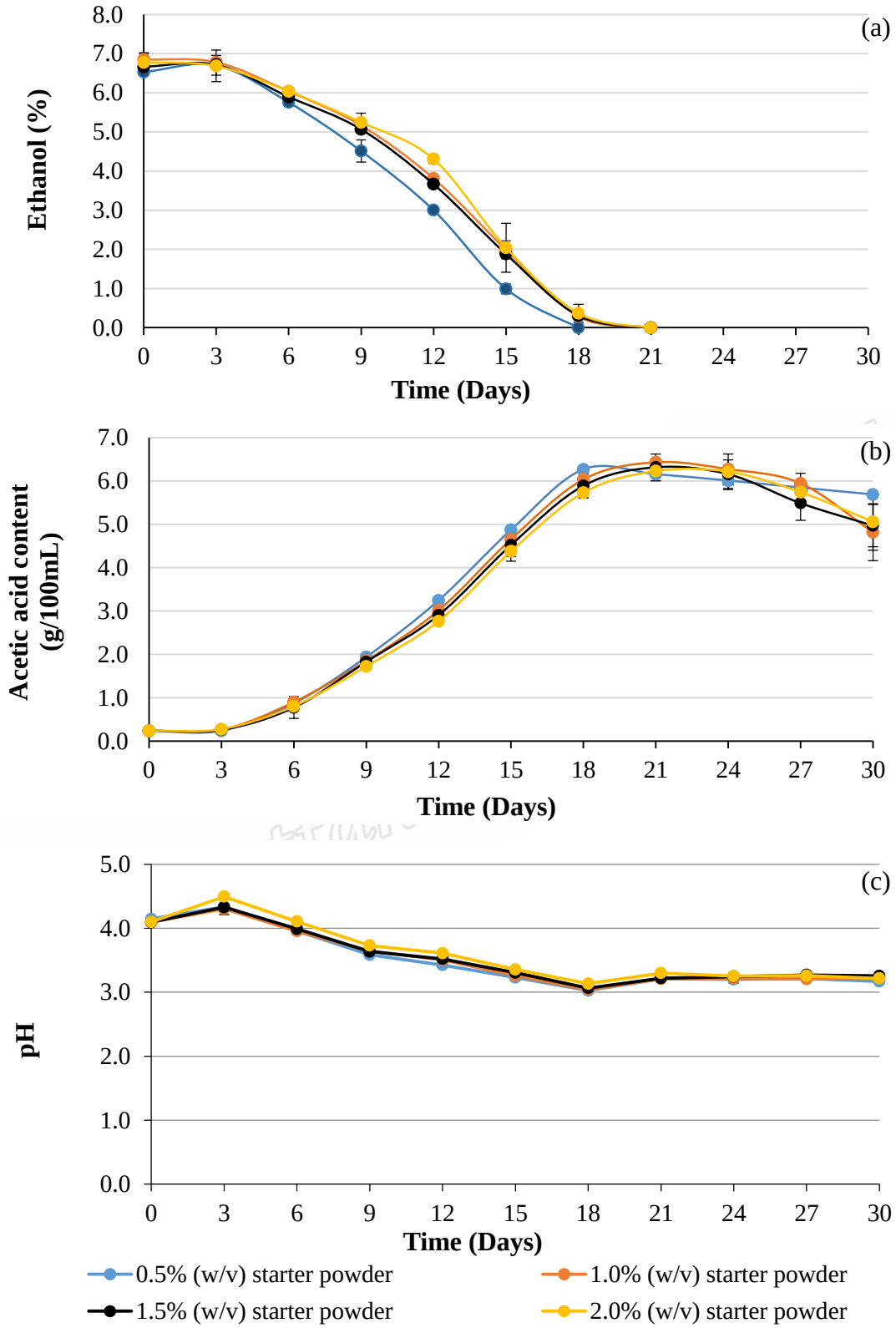

Figure 16 The ethanol content (a), acetic acid content (b) and pH change (c) of coconut wine fermented with different *A. aceti* TISTR 102 starter powder concentrations

powder produced the statistically higher acetic acid content as compared to those obtained from the other treatments at the same time. The acetic acid increased within only 18 days and the higher acid content was obtained at the lower strater powder concentration. Indeed, acetic acid 6.27±0.02 g/100 mL was recored at 0.5% (w/v) starter powder that was significantly different from 6.04±0.14, 5.89±0.27 and 5.73±0.12 (g/100mL) obtained at 1.0, 1.5 and 2.0% (w/v) starter powder. Acetic acid content stagnanted for the further time, even declined at the end of fermentation process.

Figure 16c shows the pH changes in fermention process. In all of the treatements, the pH decreased significantly within the first 18 days correspondingly when the acetic acid produced. The treatment that produced the higher acetic acid obviously had the lower pH values. From the initial pH 4.10 approximately, pH values were 3.23±0.00 , 3.26±0.01, 3.31±0.01 and 3.36±0.01 at 0.5, 1.0, 1.5 and 2.0% (w/v) starter powder, respectively. There was a slightly increase in pH thereafter but the difference was insignificant.

In the first three days, there was not any significant changes in either consumed ethanol or produced acetic acid, which reflected the adaptation (lag phase) of the starter after inoculating into the fermenting substrate. This phenomenon also represented for disruption of yeast activity by the presence of acetaldehyde that come from biological oxidation of *Acetobacter* (Claro *et al.*, 2007). Additionally, the acetic acid produced also decreased the extracellular pH that significantly affected to the intracellular pH of yeast and ethanol production also (Valli *et al.*, 2005). The exponential phase could be observed when the acetic acid increased significantly via linear trend. Obviously, there was equivalent conversion between ethanol and acetic acid when every 1% (v/v) ethanol concentration could produce approximate 1% (w/v) acetic acid content. The fermentation process obviously completed when ethanol was run out and acetic acid content stagnated simultaneously at the 18 day. The decrease in acetic acid content in the further time represented for the stationary phase of starter and moreover pointed out the overoxidation activity of *Acetobacter* strains. The slightly higher in ethanol consumption and higher acetic acid produced at treatment of 0.5% (w/v) starter powder was possibly due to the higher oxygen uptake during fermentation process. In fact that, to produce starter powder, rice bran played role as

Prince of Songkla University Pattani Campus

the carrier in which the bacteria suspension immobilized on for drying and fermentation process. So, for fermentation in static conditions, the starter powder inoculated into alcoholic substrate with more concentration had thicker sediment layer which could prevent bacteria from oxygen uptake. With 0.5% (w/v) starter powder, the acetification rate was 3.48 g/L/d; the fermentation efficiency that calculated by the ratio between produced acetic acid (w/v) and consumed ethanol concentration (w/v) was 89%. So far, several researches on vinegar fermentation have been investigated. For examples, pineapple juice was fermented through 2 stages: alcoholic fermentation with isolated yeast *S. cerevisae* (LAS01) and acetification with *Acetobacter* sp ASV03 to produce vinegar (Sossou *et al*., 2009). The alcoholic fermentation completed within 4 days and acetic acid attained 4.5 %, 5.3 Brix and pH 2.8 from 23 to 25 days in acetification. Kocher *et al*. (2006) showed that the fermentation of sugar cane juice with *S. cerevisae* could produce 8% ethanol in 48 hours. The *Acetobacter aceti* NRRL 746 was adsorbed into some fibrous materials (e.g. bagasse, corn cobs and wood shavings) and submerged into the ethanol could produce 5.9 to 6.7% acetic acid content after 28 days.

To sum up, 0.5% (w/v) starter powder was adequate to produce acetic acid more than 6.0% (w/v) efficiently within approximate 18 days. The results in this experiment were considered to be comparable to previous studies with the short fermentation time, and relatively high efficiency. Moreover, it cannot be denied the quality improvement and advantage in fermenting performance.

4.4.4 Preliminary quality evaluation of coconut vinegar

Coconut water was fermented into vinegar with baker's yeast and *A. aceti* TISTR 102 starter powder for 20 days. It was a cloudy and slightly-yellow liquid with a distinct flavor; contained 5.71±0.26 g/100 mL acetic acid content, 0.3±0.10 (% v/v) residual ethanol, 5.4±0.2 ˚Brix and pH 3.11±0.03. The control sample was traditional palm sap vinegar that contained 4.5±0.50 g/100mL acidity, 0.50±0.20 (% v/v) residual ethanol, 5.7±0.2˚Brix and pH 3.09±0.02. The two samples are shown in Figure 17; while the sensory scores of two samples are presented in Figure 18.

Appearance attribute of palm sap vinegar was rated at 7.10±1.56 that significantly preferred to the appearance of coconut vinegar with 5.77±1.54 scores. The scores of odor attribute were 6.03±1.90 and 5.19±1.80 for coconut vinegar and

palm sap vinegar, respectively. The coconut vinegar had 6.55±1.69 scores for sourness, while the sourness of palm sap vinegar attained 6.30±2.13 scores. For overall acceptance, the panelists accepted coconut vinegar and palm sap vinegar with the scores were rated at 6.48±1.23 and 6.41±1.72, respectively.

Figure 17 Coconut vinegar (left) and palm sap vinegar (right)

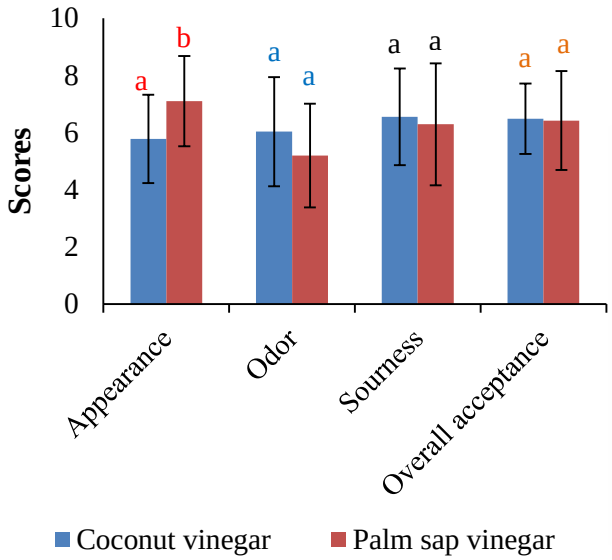

Figure 18 The sensory scores for coconut vinegar and palm sap vinegar

The high standard deviation is unavoidable in sensory evaluation when the results were done by intuition/attitude of panelists about the samples. However, the lower standard deviation possibly reflects the more reliability on the results. In spite of significant differences in scores, the panelist rated slightly higher scores for coconut vinegar (exception appearance attribute) with the lower standard deviations as compared to the counterpart. The lower score for appearance was probably due to the turbidity and lower lightness as fermenting with starter powder and its carrier (rice bran). So, coconut vinegar produced by baker's yeast and starter powder possibly was comparable to traditional palm sap vinegar in sensory qualities. In the future, the clarification treatments should be studied to improve the appearance of the final product and the other researches on application of coconut vinegar in foods also could be carried out for adding values and market expanding.

Prince of Songkla University Pattani Campus

CHAPTER 5
CONCLUSIONS AND RECOMMENDATIONS

5.1 Optimization of culture conditions for *A. aceti* TISTR 102

Coconut water was an economic and effectively comparable medium to culture *A. aceti* TISTR 102. The presence of banana juice in coconut water could enhance the growth of *A. aceti* TISTR102 and the highest cell viability at stationary phase was 8.82–9.02 log CFU/mL with 50% (v/v) added banana juice. Besides, ammonium sulfate and yeast extract did not positively influence on the bacterial growth, resulted in cost reduction for culture this bacterium. The higher shaking speed 150 rpm enhanced the cell viability with significantly viable cells as compared to those obtained at the lower shaking speed 120 rpm.

In the future, the shaking speed possibly varied in a wider range to supportively investigate the effect of shaking speed. These results also could be considered as the primary data for the further/advanced experimental design such as response surface methodology for the completely optimization experiments.

5.2 Preparation and storage of *A. aceti* TISTR 102 starter powder

Commercial sucrose performed its protective effects on the cell viability of *A. aceti* TISTR 102 with a comparably economic cost. The increase in sucrose concentrations increased the survival rate of bacteria after drying. The sucrose at 20% (w/v) could recover 79.07±3.35 (%) viable cells that significantly higher than survival rate 68.24±1.42 (%) of non-protected treatment. However, the increase in sucrose conentratration could firstly reduce the viable cells in suspension, leading to decrease in initial concentration of starter powder. Thus, the initial viable cells should be adjusted as much as possible for the resuspension step, resulted in the sufficiently initial cell concentration for efficiency fermentation.

During storage, silica gel effectively performed when it could kep the water activity of starter powder in constant and slow down the increase of water content. The cell viability, as the rsults, also could remain up to 2 months at refrigerated temperature. However, the other study on fermentative ability of the starter powder during storage should be carried out to support for the results.

Prince of Songkla University Pattani Campus

5.3 Preparation of vinegar from coconut water using baker's yeast and *A. aceti* TISTR 102 starter powder

Coconut vinegar was produced through 2 stages: alcoholic fermentation with baker's yeast and acetous fermentation with *A. aceti* TISTR 102 starter powder. The baker's yeast concentration and the sugar content had significant effects on the fermentation performance. Concretely, the increase in baker's yeast concentration decreased the fermentation time, while increase in sugar content increased the ethanol concentration produced and took longer time to complete. However, coconut water at 12% (w/v) and 0.4% (w/v) baker's yeast were adequate to produce approximately 6% (w/v) for the later acetification process. For acetification, the starter at 0.5% (w/v) sufficiently produced 6.27±0.02 g/100mL acidity after approximately 18 days, attaining 89% fermentation efficiency. The coconut vinegar was evaluated and rated comparably, even slightly higher than traditional palm sap vinegar for almost sensorial attributes.

From the results, the lower concentrations of starter powder could be investigated to completely figure out the effects of starter powder concentration on the fermentation. The sensory evaluation experiment should be continuously performed with the panelists who use or apply vinegar in cooking frequently (e.g. housewives, chefs) to get the more objective results about the vinegar qualities.

Prince of Songkla University Pattani Campus

REFERENCES

Adam, M.R. 1998. Vinegar. In Microbiology of Fermented Foods, Brian J.B. Wood, editor. Blackie Academic & Professional, London, pp. 1-47.

Akin-Osanaiye, B. C., Nzelibe, H. C. and Agbaji, A. S. 2008. Ethanol production from *Carica papaya* (pawpaw) fruit waste. Asian Journal of Biochemistry. 3(3), 188-193.

Akubor, P.I., Obio, S.O., Nwadomere, K.A. and Obiomah, E. 2003. Production and quality evaluation of banana wine. Plant Foods for Human Nutrition. 58(3), 1-6.

AOAC. 2000. Official Method of Association of Analysis. The Association of Official Analysis Chemistry. Inc. Arlington, Verginaia: Arlington.

Asli, M. S. 2010. A study on some efficient parameters in batch fermentation of ethanol using *Saccharomyces cerevesiae* SC1 extracted from fermented siahe sardasht pomace. African Journal of Biotechnology. 9(20), 2906-2912.

Belay, T. and Sonnenfeld, G. 2002. Differential effects of catecholamines on in vitro growth of pathogenic bacteria. Life Sciences. 71(4), 447-456.

Belay, T., Aviles, H., Vance, M., Fountain, K. and Sonnenfeld, G. 2003. Catecholamines and in vitro growth of pathogenic bacteria: enhancement of growth varies greatly among bacterial species. Life Sciences. 73(12), 1527-1535.

Charoenchai, C., Fleet, G.H. and Henschke, P.A. 1998. Effects of temperature, pH, and sugar concentration on the growth rates and cell biomass of wine. American Journal of Enology and Viticulture. 49(3), 283-288.

Chatterjee, G. 2009. Optimization and kinetic modelling of natural vinegar production from palm juice. Ph.D. Thesis, Faculty of Food Technology and Biochemical Engineering, Jadavpur University Kolkata.

Chávez, B. E. and Ledeboer, A. M. 2007. Drying of probiotics: optimization of formulation and process to enhance storage survival. Drying Technology. 25(7-8), 1193-1201.

Ciani, M., Ferraro, L. and Fatichenti, F. 2000. Influence of glycerol production on the aerobic and anaerobic growth of the wine yeast *Candida stellata*. Enzyme and Microbial Technology. 27(9), 698-703.

Claro, F. B., Rijsbrack, K. and Soares, E. V. 2007. Flocculation onset in *Saccharomyces cerevisiae*: effect of ethanol, heat and osmotic stress. Journal of Applied Microbiology. 102(3), 693-700.

Conner, H.A. and Allgeier, R.J. 1976. Vinegar: its history and development. In Advances in Applied Microbiology, volume 20th, D. Perlman, editor. Academic press, U.S.A, pp, 87.

Corcoran, B.M., Ross, R.P., Fitzgerald, G.F. and Stanton, C. 2004. Comparative survival of probiotic lactobacilli spray-dried in the presence of prebiotic substances. Journal of Applied Microbiology. 96(5), 1024-1039.

De Ley, J. and Schell, J. 1959. Oxidation of several substrates by *Acetobacter aceti*. Journal of Bacteriology. 77(4), 445-451.

Dianawati, D., Mishra, V. and Shah, N. P. 2013. Stability of microencapsulated *Lactobacillus acidophilus* and *Lactococcus lactis* ssp. *cremori*s during storage at room temperature at low a_w. Food Research International. 50(1), 259-265.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. 1956. Colorimetric method of sugars and related substances. Analytical Chemistry. 28, 350-356

Freestone, P. P. E., Haigh, R. D. and Lyte, M. 2009. Bacterial growth enhancer. U.S. Patent No. 20,090,087,517. Washington, DC: US. Patent and Trademark Office.

Friesen, T., Hill, G., Pugsley, T., Holloway, G. and Zimmerman, D. 2005. Experimental determination of viability loss of *Penicillium bilaiae* conidia during convective air-drying. Applied Microbiology and Biotechnology. 68(3), 397-404.

Fu, N. and Chen, X.D. 2011. Towards a maximal cell survival in convective thermal drying processes. Food Research International. 44(5), 1127-1149.

Gardiner, G. E., Bouchier, P., O'Sullivan, E., Kelly, J., Collins, J. K., Fitzgerald, G. and Stanton, C. 2002. A spray-dried culture for probiotic Cheddar cheese manufacture. International Dairy Journal. 12(9), 749-756.

Gerbi, V., Zeppa, G., Antonelli, A., and Carnacini, A. 1997. Sensory characterization of wine vinegars. Food Quality and Preference. 8(1), 27-34.

Giovanelli, G., Peri, C. and Parravicini, E. 1996. Kinetics of grape juice fermentation under aerobic and anaerobic conditions. American Journal of Enology and Viticulture. 47(4), 429-434.

Grahovac, J., Dodić, J., Jokić, A., Dodić, S. and Popov, S. 2012. Optimization of ethanol production from thick juice: A response surface methodology approach. Fuel. 93, 221-228.

Guillamón, J.M. and Mas, A. 2009. Acetic acid bacteria. In Biology of Microorganisms on Grapes, in Must and in Wine, H. König, G. Unden and J. Fröhlich, editors. Springer, Berlin Heidelberg, pp. 31-46.

Gullo, M. and Giudici, P. 2008. Acetic acid bacteria in traditional balsamic vinegar: phenotypic traits relevant for starter cultures selection. International Journal of Food Microbiology. 125(1), 46-53.

Gullo, M., Caggia, C., De Vero, L. and Giudici, P. 2006. Characterization of acetic acid bacteria in "traditional balsamic vinegar". International Journal of Food Microbiology. 106(2), 209-212.

Heldman, D. R. and Hartel, R. W. 1997. Food dehydration. In Principles of Food Processing, D.R.Heldman and R.W. Hartel, editors. Aspen Pulisher, USA, pp. 177-218

Holzapfel, W. 1997. Use of starter cultures in fermentation on a household scale. Food Control. 8(5), 241-258.

Holzapfel, W. 2002. Appropriate starter culture technologies for small-scale fermentation in developing countries. International Journal of Food Microbiology. 75, 197-212.

Horiuchi, J.I, Kanno, T. and Kobayashi, M. 1999. New vinegar production from onions. Journal of Bioscience and Bio-engineering. 88(1), 107-109.

Hutkins, R. W. 2008. Microbiology and Technology of Fermented Foods, Blackwell Publishing, Chicago, U.S.A., pp, 397.

Jackson, J.C., Gordon, A., Wizzard, G., McCook, K. and Rolle, R. 2004. Changes in chemical composition of coconut (*Cocos nucifera*) water during maturation of the fruit. Journal of the Science of Food and Agriculture. 84(9), 1049-1052.

Kim, S.H., Cho, H.K. and Shin, H.S. 2012. Physicochemical properties and antioxidant activities of commercial vinegar drinks in Korea. Food Science and Biotechnology. 21(6), 1729-1734.

Kinney, K. S., Austin, C. E., Morton, D. S. and Sonnenfeld, G. 2000. Norepinephrine as a growth stimulating factor in bacteria - mechanistic studies. Life Sciences. 67(25), 3075-3085.

Kocher, G. S., Dhillon, H. K. and Joshi, N. 2014. Scale up of sugarcane vinegar production by recycling of successive fermentation batches and its organoleptic evaluation. Journal of Food Processing and Preservation. 38(3), 955-963.

Kocher, G. S., Kalra, K. L. and Phutela, R. P. 2006. Comparative production of sugarcane vinegar by different immobilization techniques. Journal of the Institute of Brewing. 112(3), 264-266.

Kumoro, A.C., Rianasari, D., Pinandita, A.P.P., Retnowati, D.S. and Budiyati, C.S. 2012. Preparation of wine from jackfruit (*Artocarpus heterophyllus lam*) juice using baker's yeast: Effect of yeast and initial sugar concentrations. World Applied Sciences Journal. 16(9), 1262-1268.

Lasko, D. R., Schwerdel, C., Bailey, J. E. and Sauer, U. 1997. Acetate-Specific Stress Response in Acetate-Resistant Bacteria: An Analysis of Protein Patterns. Biotechnology progress. 13(5), 519-523.

Lee, W.C., Yusof, S., Hamid, N.S.A. and Baharin, B.S. 2006. Optimizing conditions for hot water extraction of banana juice using response surface methodology (RSM). Journal of Food Engineering. 75(4), 473-479.

Leslie, S. B., Israeli, E., Lighthart, B., Crowe, J. H. and Crowe, L. M. 1995. Trehalose and sucrose protect both membranes and proteins in intact bacteria during drying. Applied and Environmental Microbiology. 61(10), 3592-3597.

Lin, Y., Zhang, W., Li, C., Sakakibara, K., Tanaka, S. and Kong, H. 2012. Factors effecting ethanol fermentation using *Saccharomyces cerevisiae* (BY4742). Biomass and Bioenergy. 47, 395-401.

Loesecke, H.V. 1929. Preparation of banana vinegar. Industrial and Engineering Chemistry. 21(2), 175-176.

Lyte, M. 1997. Induction of Gram-negative bacterial growth by neurochemical containing banana (*Musa* x *paradisiaca*) extracts. FEMS Microbiology Letters. 154(2), 245-250.

Macauley, S., McNeil, B. and Harvey, L. M. 2001. The genus *Gluconobacter* and its applications in biotechnology. Critical Reviews in Biotechnology. 21(1), 1-25.

Mamlouk, D. and Gullo, M. 2013. Acetic acid bacteria: physiology and carbon sources oxidation. Indian Journal of Microbiology. 54(4), 1-8.

Matloob, M. H. 2014. Zahdi Date Vinegar: Production and Characterization. American Journal of Food Technology. 9(5), 231-245.

Miller, G. L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry. 31(3), 426-428.

Morgan CA, Herman N, White PA, Vesey G. 2006. Preservation of Micro-organisms by drying. Journal of Microbiological 66: 183-193.

Müller, T., Walter, B., Wirtz, A. and Burkovski, A. 2006. Ammonium toxicity in bacteria. Current Microbiology. 52(5), 400-406.

Nagodawithana, T. W., Castellano, C. and Steinkraus, K. H. 1974. Effect of dissolved oxygen, temperature, initial cell count, and sugar concentration on the viability of *Saccharomyces cerevisiae* in rapid fermentations. Applied Microbiology. 28(3), 383-391.

Ndip, R. N., Akoachere, J. F., Dopgima, L. L. and Ndip, L. M. 2001. Characterization of yeast strains for wine production. Applied Biochemistry and Biotechnology. 95(3), 209-220.

Ndoye, B., Cleenwerck, I., Destain, J., Guiro, A. T. and Thonart, P. 2009. Preservation of vinegar acetic acid bacteria. In Vinegars of the World, L. Solieri and P. Giudici, editors. Springer Milan, USA, pp. 61-71.

Ndoye, B., Weekers, F., Diawara, B., Guiro, A.T. and Thonart, P. 2007. Survival and preservation after freeze-drying process of thermoresistant acetic acid bacteria isolated from tropical products of Subsaharan Africa. Journal of Food Engineering. 79(4), 1374-382.

Nomura, Y., Yamamoto, M., Matsushita, K. and Kumagai, H. 1998. Preparation and preservation of freeze-dried cells of acetic acid bacteria with aldehyde oxidase activity. Bioscience, Biotechnology and Biochemistry. 62(6), 1134-1137.

Ocloo, F. C. K. and Ayernor, G. S. 2010. Production of alcohol from cassava flour hydrolysate. Journal of Brewing and Distilling. 1(2), 15-21.

Oldenhof, H., Wolkers, W. F., Fonseca, F., Passot, S. and Marin, M. 2005. Effect of Sucrose and Maltodextrin on the Physical Properties and Survival of Air-Dried *Lactobacillus bulgaricus*: An in Situ Fourier Transform Infrared Spectroscopy Study. Biotechnology Progress. 21(3), 885-892.

Onwuka, U. and Awam, F.N. 2001. The potential for baker's yeast (*Saccharomyces cerevisiae*) in the production of wine from banana, cooking banana and plantain. Food Service Technology. 1(3-4), 127-132.

Palmfeldt, J., Rådström, P. and Hahn-Hägerdal, B. 2003. Optimization of initial cell concentration enhances freeze-drying tolerance of *Pseudomonas chlororaphis*. Cryobiology. 47(1), 21-29.

Pereira, A.P., Mendes-Ferreira, A., Oliveira, J.M., Estevinho, L.M. and Mendes-Faia, A. 2013. High-cell-density fermentation of *Saccharomyces cerevisiae* for the optimization of mead production. Food Microbiology. 33(1), 114-123.

Prades, A., Dornier, D., Diop N. and Pain J.P. 2012. Coconut water uses, composition and properties: a review. Fruits. 67(2), 87-107.

Punchihewa, P.G. and Arancon, R.N. 2006. Coconut: postharvest operations. Asian and Pacific Coconut Community, website: http://www.apcc.org.sg, and FAO, website: http://www.fao.org.

Ray, B. 2004. Fundamental Food Microbiology, 3rd ed, CRC press, Boca Raton, Florida, USA, pp. 132-133, 187.

Rodrigues, F., Ludovico, P. and Leão, C. 2006. Sugar metabolism in Yeasts: an Overview of Aerobic and Anaerobic Glucose Catabolism. Biodiversity and Ecophysiology of Yeasts, G.Péter and C. Rosa, editors. Springer Berlin Heidelberg, Germany, pp. 101-121.

Santivarangkna, C., Higl, B. and Foerst, P. 2008. Protection mechanisms of sugars during different stages of preparation process of dried lactic acid starter cultures. Food Microbiology. 25(3), 429-441.

Schlepütz, T., Gerhards, J.P. and Büchs, J. 2013. Ensuring constant oxygen supply during inoculation is essential to obtain reproducible results with obligatory aerobic acetic acid bacteria in vinegar production. Process Biochemistry. 48, 398–405

Seesuriyachan, P., Kuntiya, A., and Techapun, C. 2011. Exopolysaccharide production by *Lactobacillus confusus* TISTR 1498 using coconut water as an alternative carbon source: the effect of peptone, yeast extract and beef extract. Sonklanakarin Journal of Science and Technology. 33(4), 379.

Shivakumar, S. and Vijayendra, S. V. N. 2006. Production of exopolysaccharides by *Agrobacterium* sp. CFR-24 using coconut water–a byproduct of food industry. Letters in Applied Microbiology. 42(5), 477-482.

Singh, O. V., Kapur, N. and Singh, R. P. 2005. Evaluation of agro-food byproducts for gluconic acid production by *Aspergillus niger* ORS-4.410. World Journal of Microbiology and Biotechnology. 21(4), 519-524.

Solieri, L. and Giudici, P. 2008. Yeasts associated to traditional balsamic vinegar: ecological and technological features. International Journal of Food Microbiology. 125(1), 36-45.

Solieri, L. and Giudici, P. 2009. Vinegars of the World. In Vinegars of the World. L. Solieri and P. Giudici, editors. Springer Milan, USA, pp. 1-16.

Sossou, S. K., Ameyapoh, Y., Karou, S. D. and Souza, C. D. 2009. Study of pineapple peelings processing into vinegar by biotechnology. Pakistan Journal of Biological Sciences. 12(11), 859-865.

Supakod, S. and Wongwicharn, A. 2012. Cheap media for inoculum preparation of acetic acid bacteria. Advanced Materials Research. 506, 575-578.

Tan, T.C., Cheng, L.H., Bhat, R., Rusul, G. and Easa, A.M. 2013. Composition, physicochemical properties and thermal inactivation kinetics of polyphenol oxidase and peroxidase from coconut (*Cocos nucifera*) water obtained from immature, mature and overly mature coconut. Food Chemistry. 142, 121-128.

Tantratian, S., Tammarate, P., Krusong, W., Bhattarakosol, P. and Phunsri, A. 2005. Effect of dissolved oxygen on cellulose production by *Acetobacter* sp. J. Sci. Res. Chula Univ. 30, 179-186.

Teixeira, P., Castro, H. and Kirby, R. 1995. Spray drying as a method for preparing concentrated cultures of Lactobacillus bulgaricus. Journal of Applied Bacteriology. 78(4), 456-462.

Tesfaye, W., Morales, M.L., García-Parrilla, M.C. and Troncoso, A.M. 2000. Set up and optimization of a laboratory scale fermentor for the production of wine vinegar. Journal of the Institute of Brewing. 106(4), 215-219.

Tymczyszyn, E.E., Gómez-Zavaglia, A. and Disalvo, E.A. 2007. Effect of sugars and growth media on the dehydration of *Lactobacillus delbrueckii* ssp. *bulgaricus*. Journal of Applied Microbiology. 102(3), 845-851.

Unagul, P., Assantachai, C., Phadungruengluij, S., Suphantharika, M., Tanticharoen, M. and Verduyn, C. 2007. Coconut water as a medium additive for the production of docosahexaenoic acid (C22:6n3) by *Schizochytrium mangrovei* Sk-02. Bioresource Technology. 98, 281-287.

Valli, M., Sauer, M., Branduardi, P., Borth, N., Porro, D.and Mattanovich, D. 2005. Intracellular pH distribution in Saccharomyces cerevisiae cell populations, analyzed by flow cytometry. Applied and Environmental Microbiology. 71(3), 1515-1521.

Vega, E. Z., Glatz, B. A. and Hammond, E. G. 1988. Optimization of banana juice fermentation for the production of microbial oil. Applied and Environmental Microbiology. 54(3), 748-752.

Victor, E. 2013. *Cocos nucifera* (Coconut) fruit: A review of its medical properties. Advances in Agriculture, Sciences and Engineering Research. 3(3), 718-723.

Vithlani, V.A. and Patel, H.V. 2010. Production of functional vinegar from Indian Jujube (*Zizyphus mauritiana)* and its antioxidant properties. Journal of Food Technology. 8(3), 143-149.

Walker, G.M. 1998. Yeast metabolism. In Yeast Physiology and Biotechnology, G.M. Walker, editor. John Wiley and Sons, England, pp. 208-255

Wongsudaluk, W. 2012. Production of *Acetobacter aceti* TISTR 102 Starter Power by Low-Temperature Thermal Drying. MSc Thesis, Food Science and Nutrition, Faculty of Science and technology, Prince of Songkla University, Thailand.

Yong, J.W.H., Ge, L., Fei, Ng.Y. and Tan, S.N. 2009. The chemical composition and biological properties of coconut (*Cocos nucifera* L.). Water Molecules. 14(12), 5144-5164.

Ying, D. Y., Phoon, M. C., Sanguansri, L., Weerakkody, R., Burgar, I. and Augustin, M. A. 2010. Microencapsulated *Lactobacillus rhamnosus* GG powders: relationship of powder physical properties to probiotic survival during storage. Journal of Food Science. 75(9), E588-E595.

Website

http://www.agr.gc.ca/eng/programs-and-services/list-of-programs-and-services/agri-food-trade-service/?id=1410965065217 [accessed on 15th October, 2013]

APPENDICES

Prince of Songkla University
Pattani Campus

Appendix A

Microbial culture media

1. **Glucose Yeast Extract Broth (GY broth)** (Wongsudaluk, 2012)

Compositions	Glucose	100.0	g
	Yeast extract	10.0	g
	Distilled water	1000	L

Sterilization was performed by autoclaving at 121°C for 15 minutes

2. **Glucose Yeast Extract Agar (GYC agar)** (Wongsudaluk, 2012)

Compositions	Glucose	100.0	g
	Yeast extract	10.0	g
	Calcium carbonate	10.0	g
	Agar	20.0	g
	Distilled water	1000	mL

Sterilization was performed by autoclaving at 121°C for 15 min.

The medium can be stored at 4°C for up to one month or at room temperature for up to one week.

3. **Yeast Extract Peptone Dextrose Agar (YEPD agar)** (Wongsudaluk, 2012)

Compositions	Glucose	20.0	g
	Yeast extract	10.0	g
	Peptone	20.0	g
	Agar	20.0	g
	Distilled water	1000	mL

Sterilization was performed by autoclaving at 121°C for 15 minutes.

The medium can be stored at 4°C for up to one month or at room temperature for up to one week.

Prince of Songkla University Pattani Campus

Appendix B

Methodology/Reagents/Chemicals

1. **Reducing sugar: 3, 5-dinitrosalicylic acid (DNS) method** (Miller, 1959)

Chemical reagents	3, 5-dinitrosalicylic acid	10.0	g
	Phenol	2.0	g
	Sodium sulfite	0.5	g
	Sodium hydroxide	10.0	g
	Potassium sodium tartrate	200.0	g
	Distilled water	1000	mL

Procedure

- The sample 1.0 mL was mixed with 3.0 mL of DNS solution. The mixture was boiled for 5 minutes.
- The sample was cooled down by immersing the sample tube into cold water immediately. Six (6) mL of water is added. The mixture was mixed well with vortex mixer and measured at absorbance 550 nm.

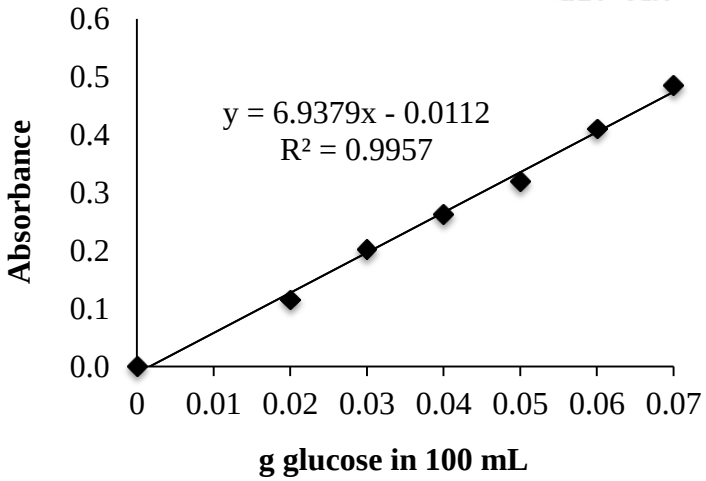

Figure 19 The standard curve for 3, 5-DNS method

- Absorbance 550 nm was converted to glucose concentration with standard curve.

Standard curve

- Glucose with 0.1 g was diluted into 100 mL volumetric flask as the stock solution at concentration 0.1 g in 100 m. The serial dilutions 0.02, 0.03, 0.04, 0.05 and 0.06 g/100 mL were prepared by taking 2, 3, 4, 5 and 6 mL of stock solution and adjusting into 10 mL with distilled water in volumetric flasks. The blank was prepared without glucose solution
- Following the above procedure, samples were measured at 550 nm with 3 replications for each dilution
- Plot the glucose concentrations in the horizontal axis and recorded absorbance in vertical axis to establish the standard graph in Figure 19

2. Total sugar: Phenol sulfuric acid method (Dubois *et al*., 1956)

Chemical reagents	Sufuric acid	98%
	Phenol	5.0%

Procedure

- The sample 0.5 mL was mixed with 0.5 mL of phenol solution.
- The mixture was mixed with concentrated sulfuric acid 2.5 mL. The samples was mixed well with vortex mixer, cooled for 30 minutes and measured at absorbance 490 nm.
- Absorbance 490 nm was converted to glucose concentration with standard curve.

Standard curve

- Glucose with 0.1 g was diluted into 1000 mL volumetric flask as the stock solution at concentration 0.01 g in 100 mL.
- The serial dilutions 0.002, 0.003, 0.004, 0.005 and 0.006 g/100 mL were prepared by taking 2, 3, 4, 5 and 6 mL of stock solution and adjusting into 10 mL with distilled water in volumetric flasks. The blank was prepared without glucose solution.

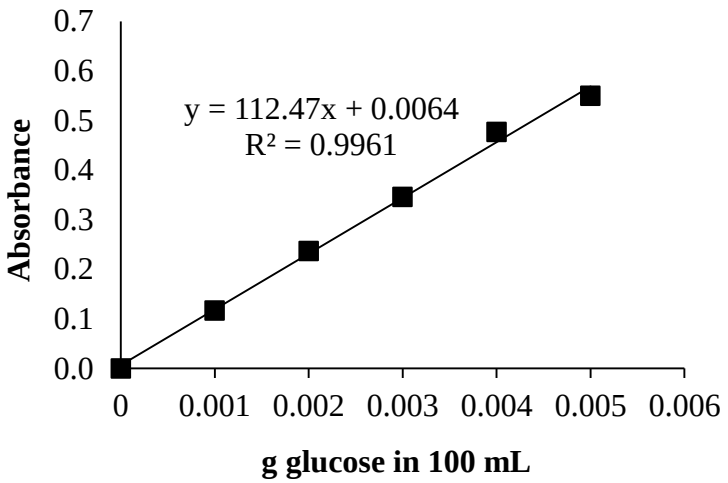

Figure 20 The standard curve for phenol sulfuric acid method

- Following the above procedure, samples were measured at 490 nm with 3 replications for each dilution.
- Plot the glucose concentrations in the horizontal axis and recorded absorbance in vertical axis to establish the standard graph in Figure 20

3. Ethanol determination using Ebulliometer (Wongsudaluk, 2012)

Principle

The boiling temperature of wines depends on their alcoholic content and the atmospheric pressure. The boiling temperatures of the water and wine to be tested are written and a calculation disk is used to find the alcoholic strength with an accuracy of 0.1% volume

Procedure

- Empty the boiler and rinse it with samples to be tested.
- Pour a full measure of sample (using the glass cylinder) into the boiler; fill the cooling tank with cold water and place the thermometer.
- Heat the sample and the mercury will raise and the stop after about 5 minutes. Wait about 30 seconds and then read the temperature.
- Put off the lamp, empty the device and continue with the next samples
- Do the same above procedure for water to measure the water boiling point, but without water in the cooling tank.

Directions for using the Ebulliometer disk

- Read the water boiling temperature and bring the circular sliding part round until the division is directly opposite the 0 division of the fixed graduations on either side.
- Hold the square plate, after reading the ebullition degree of the samples that have been tested, then read the opposite this degree (thermometer) the alcoholic degree of the samples in % volume.

Prince of Songkla University Pattani Campus

4. Acidity determination by titration method with 0.1N NaOH (AOAC, 2000)

Chemical reagents

Deionized distilled water	Distilled water was boiled within 20 minutes		
0.1N Sodium hydroxide	Sodium hydroxide	40.0	g
	Distilled water	1000	mL
Phenolphthalein solution	Phenolphthalein	1.0	g
	Ethanol 95%	100	mL
Standardization	Potassium Hydrogen Phthalate		

Standardization for 0.1N sodium hydroxide solution

- Potassium hydrogen phthalate was dried in oven at 120^{o}C for 2 hours and let cool down in desiccator.
- Weight 0.3 g dried potassium hydrogen phthalate and dissolve with 100 mL deionized water.
- Add 3 drops of phenolphthalein into the mixture
- Titrate the mixture with sodium hydroxide until reaching the pinkish end point
- The normality (N) of sodium hydroxide solution was calculated by equation

$$N_{NaOH} = \frac{1000 \text{ x } g_{KHC8H4O4}}{204.229 \text{ x } mL_{NaOH}}$$

Procedure

- Take 1 mL of sample and mix sample with 5 mL of deionized water
- Add 3 drops of phenolphthalein into the mixture
- Titrate the mixture with 0.1N sodium hydroxide until reaching the pinkish end point
- The acidity (g acetic acid in 100 mL) was calculated by equation

$$\text{Acidity} = \frac{N \text{ x } V_1 \text{ x } 60.1 \text{ x } 100}{1{,}000 \text{ x } V_2}$$

Where N the normality of sodium hydroxide solution (N)

V_1 the volume of titrated sodium hydroxide (mL)

V_2 the volume of sample (mL)

60.1 molecular weight of acetic acid

Prince of Songkla University Pattani Campus

5. Sensory evaluation form

SENSORY EVALUATION FORM

Name: …….

Date: …….……Time……...

Instructions:

- Please, taste these samples (vinegars) and use the appropriate scale to show your attitude (like/ dislike) by checking at the point that best describe your feelings about them.
- Hedonic rating scale:

1-dislike extremely	2-dislike very much	3-dislike moderately
4-dislike slightly	5-neither like nor dislike	6-like slightly
7-like moderately	8-like very much	9-like extremely

Attributes	Sample ……..	Sample ……	Sample ……
Appearance (color, turbidity) (ลักษณะปรากฏ เช่น สี, ความขุ่น)			
Odor (กลิ่น/ กลิ่นเหม็นชื่อ)			
Sourness/ Acidity (เปรี้ยว / ความเป็นกรด)			
Overall acceptance (ความชอบโดยรวม)			

Remarks

……

……

……

Thank you

Prince of Songkla University Pattani Campus

6. Moisture content (AOAC, 2000**)**

Equipment: Moisture cans, digital balance, desiccator and oven

Procedure

- Dry moisture cans in hot air oven at 105^{o}C for 3 hours and let them cool down in desiccator
- Weight the dried empty moisture cans and record as W_c
- Weight samples into the moisture cans with approximately 2.00±0.01 g and record as W_i
- Dry the moisture cans containing samples in hot air oven at 105^{o}C and monitor the weight every hour until the weight to be constant (±0.003). Record the data as W_f
- Calculate the moisture content in wet basis by the following equation

$$\text{MC (\%)} = \frac{W_f - W_c}{W_i - W_c} x100$$

7. Preparation of phosphate buffer 50 mM 6.5 pH (Wongsudaluk, 2012)

Solution A: Sodium dihydrogen phosphate dihydrate ($NaH_2PO_4.2H_2O$) 1M

Sodium dihydrogen phosphate	156.01	g
Distilled water	1,000	mL

Solution B: Di-sodium hydrogen phosphate dihydrate ($Na_2HPO_4.2H_2O$) 1M

Di-sodium hydrogen phosphate dehydrate	177.99	g
Distilled water	1,000	mL

Mixing 685 mL of solution A and with 315 mL of solution B in 1 L volumetric flask to obtain the 1M phosphate buffer stock solution pH 6.5. The stock solution was diluted into 50mM before using for cell purification.

Prince of Songkla University Pattani Campus

Appendix C

Pictures/Illustrations

Figure 21 Namwa banana (*Musa sapientum*) with yellow and slightly brown spot

Figure 22 *A. aceti* TISTR 102 starter powder in vacuum aluminum-sealed pouch and packed with silica gel

Figure 23 Rice bran before (left) and after (right) autoclaving for sterilization

Figure 24 Commercial baker's yeast - Angel Yeast Gold Baker's

Appendix D

Data

Table 7 The cell viability (log CFU/mL) of *A. aceti* TISTR 102 in different culture media

Time (hr)	0	6	12	18	24	30	36	42	48	60	72
GYE	6.79±0.14 a, A	7.79±0.90 a, C	8.02±0.90 a, D	8.01±0.90 a, D	7.86±0.90 a, C	7.81±0.89 a, C	7.80±0.89 a, C	7.72±0.89 a, C	7.71± 0.89 a, C	7.72±0.89 a, C	7.54±0.88 a, B
Coconut water	6.79±0.06 a, A	7.74±0.10 a, B	8.01±0.11 a, C	8.17±0.06 a ,b, D	8.42±0.04 b, E	8.41±0.02 b, E	8.42±0.04 b, E	8.40±0.03 b, E	8.41±0.04 b, E	8.37± 0.08 b, E	8.33±0.06 b, E
Added 25% (v/v) banana juice	6.80±0.06 a, A	7.74±0.13 a, B	8.09±0.19 a, b, C	8.29±0.10 a ,b, C, D	8.48±0.00 b, D, E	8.61±0.25 b, C,E,F	8.76±0.08 c, F	8.803±0.15 c, F	8.80±0.16 c, F	8.77±0.04 c, d, F	8.84±0.07 c, F
Added 50% (v/v) banana juice	6.80±0.02 a, A	7.70±0.24 a, B	8.36±0.25 b, C	8.50±0.29 b, C	8.82±0.09 c, D, E	8.88±0.113 c, D, E	8.90±0.11 d, D, E	9.07±0.11 d, D, E	8.94±0.08 c, D, E	8.85±0.09 d, D, E	8.75±0.11 c, D
Added 75% (v/v) banana juice	6.80±0.02 a, A	7.74±0.13 a, B	8.22±0.06 a, b, C	8.30±0.33 a, b, C	8.62±0.11 d, D	8.70±0.16 c, D	8.83±0.04 c, d, D	8.80±0.10 c, D	8.80±0.12 c, D	8.68±0.05 c, D	8.70±0.07 c, D
Added 100% (v/v) banana juice	6.81±0.04 a, A	7.67±0.06 a, B	8.15±0.13 a, b, C	8.27±0.22 a, b, C	8.65±0.06 d, D	8.70±0.11 c, D	8.85±0.09 c, d, D	8.77±0.11 c, D	8.74±0.14 c, D	8.68±0.15 c, D	8.68±0.19 c, D

Mean values (n=3) in each column and row are followed by different normal and capital superscript letters, resepctively ($p \leq 0.05$)

Prince of Songkla University Pattani Campus

Table 8 The reducing sugar content (g/100mL) in different culture media during incubation time as culturing *A. aceti* TISTR 102

Time (hr)	**0**	**6**	**12**	**18**	**24**	**30**	**36**	**42**	**48**	**60**	**72**
GYE	21.58±0.85 A	18.15±0.3 B	18.24±0.4 B	18.46±0.3 B	17.07±0.84 C	16.70±0.30 C	16.34±0.6 C	15.32±0.7 D	15.14±0.40 D, E	14.55±0.6 D, E	14.24±0.2 D, E
Coconut water	6.48±0.69 A	6.44±0.13 A, B	6.50±0.2 A	5.85±0.52 B, C	5.71±0.06 C	5.94±0.24 A, B, C	5.79±0.20 C	5.68±0.27 C	5.37±0.38 C, D	4.80±0.22 D	4.90±0.15 D
Added 25% (v/v) banana juice	9.63±0.21 A	9.32±0.11 A, B, C	9.28±0.4 A, B	8.84±0.23 A, B, C	8.42±0.12 B, C, D	8.41±0.29 C, D	8.39±0.17 D, E	8.34±0.20 D, E	8.33±0.13 D,E	7.37±0.29 E	6.96±0.11 E
Added 50% (v/v) banana juice	11.50±0.1 A	11.02±0.3 A, B, C	11.18±0.54 A, B	10.94±0.3 A, B, C	10.43±0.38 B, C, D	10.22±0.62 C, D	10.03±0.0 D, E	9.60±0.56 D, E	9.88±0.42 E	9.21±0.11 E	9.23±0.89 E
Added 75% (v/v) banana juice	13.64±0.2 A	12.35±0.2 B	12.07±0.1 B	11.74±0.7 B, C	11.71±0.12 B, C, D	11.68±0.74 B, C, D	11.52±0.1 B, C, D, E	11.05±0.7 C, D, E	10.73±0.79 D, E	10.60±0.54 E	9.60± 0.60 F
Added 100% (v/v) banana juice	14.62±0.44 A	13.46±0.6 B	12.99±0.1 B	13.02±0.6 B	13.00±0.58 B	13.11±0.28 B	12.84±0.3 B, C	11.99±0.5 C, D	11.37±0.48 D	11.22±0.5 D	11.21±0.6 D

Mean values (n=3) in each column and row are followed by different normal and capital superscript letters, resepctively ($p \leq 0.05$)

Prince of Songkla University Pattani Campus

Table 9 The pH values of different culture media during incubation time as culturing *A. aceti* TISTR102

Time (hr)	0	6	12	18	24	30	36	42	48	60	72
GYE	4.70±0.02 a, A	4.39±0.02 a, B	4.08±0.0 a, C	3.76±0.0 a, D	3.65±0.01 a, E	3.60±0.01 a, F	3.60±0.01 a, F	3.55±0.01 a, G	3.54±0.01 a, G, H	3.53±0.00 a, H	3.51±0.01 a, I
Coconut water	4.92±0.0 c, A	4.72±0.01 d, B	4.57±0.0 e, C	4.43±0.0 b, c, D	4.39±0.02 c, d, D, E	4.36±0.02 d, D, E	4.28±0.02 d, E, F	4.22±0.02 d, F	4.15±0.0 c, F, G	4.05±0.04 c, G	3.93±0.01 b, H
Added 25% (v/v) banana juice	4.85± 0.02 b, A	4.66±0.02 c, B	4.50±0.02 c, C	4.44± 0.01 c, D	4.41±0.01 d, E	4.37±0.01 d, F	4.33± 0.02 e, G	4.29±0.01 e, H	4.19±0.0 d, I	4.05±0.03 c, J	3.92±0.01 c, K
Added 50% (v/v) banana juice	4.84±0.0 b, A	4.61±0.03 b, B	4.43±0.0 b, C	4.39± 0.01 b, D	4.28±0.02 b, E	4.19±0.01 b, F	4.05±0.01 b, G	3.94±0.02 b, H	3.87±0.0 b, I	3.76±0.02 b, J	3.69±0.01 a, b, K
Added 75% (v/v) banana juice	4.82±0.0 b, A	4.59±0.01 b, B	4.48±0.0 c, C	4.43±0.0 b, c , D	4.30±0.03 b, E	4.19±0.02 b, F	4.07±0.02 b, c, G	3.96±0.00 b, H	3.87±0.0 b, I	3.75± 0.02 b, J	3.67±0.01 a, b, K
Added 100% (v/v) banana juice	4.85± 0.02 b, A	4.61±0.01 b, B	4.53±0.0 d, C	4.45±0.05 c, D	4.37±0.03 c, E	4.24±0.03 c, F	4.10±0.03 c, G	3.99±0.02 c , H	3.88±0.0 b, I	3.75±0.02 b, J	3.67±0.01 c, K

Mean values (n=3) in each column and row are followed by different normal and capital superscript letters, resepctively ($p \leq 0.05$)

Prince of Songkla University Pattani Campus

Table 10 The cell viability (log CFU/mL) of *A. aceti* TISTR 102, reducing sugar content (g/100mL) and pH values as culturing *A. aceti* TISTR 102 in coconut water with 50 % (v/v) banana juice medium at different concentrations of ammonium sulfate

Time (hr)		**0**	**6**	**12**	**18**	**24**	**30**	**36**	**42**	**40**	**60**	**72**
	Control	7.09±0.01 a, A	7.63±0.05 a, B	8.24±0.07 a, C	8.68±0.02 a, D, E	8.70±0.07 a, D, E	8.76±0.07 a, E	8.77±0.06 a, E	8.74±0.06 b, E	8.68±0.07 a, b, D, E	8.69±0.07 b, D, E	8.60±0.08 a, b D
Cell viability	0.1% (w/v)	7.09±0.02 a, A	7.69±0.09 a, b, B	8.29±0.05 a, b, C	8.67±0.03 a, D	8.73±0.08 a, D	8.71±0.06 a, D	8.72±0.07 a, D	8.72±0.03 b, D	8.69±0.06 a, b, D	8.70±0.04 b, D	8.67±0.02 b, D
(log CFU/mL)	0.3% (w/v)	7.07±0.07 a, A	7.72±0.02 a, b, B	8.38±0.08 b, C	8.68±0.06 a, D, E	8.68±0.11 a, D, E	8.68±0.08 a, D, E	8.77±0.06 a, E	8.74±0.02 b, D, E	8.76±0.02 b, E	8.63±0.01 b, D	8.67±0.08 b, D, E
	0.5% (w/v)	7.09±0.04 a, A	7.78±0.06 b, B	8.28±0.04 a, b, C	8.62±0.08 a, E	8.64±0.02 a, E	8.65±0.01 a, E	8.68±0.05 a, E	8.63±0.02 a, E	8.63±0.03 a, E	8.53±0.08 a, D	8.52±0.05 a, D
	Control	6.60±0.15 a, A	5.87±0.10 a, B	5.72±0.02 a, B, C	5.63±0.00 a, C	5.65±0.02 a, C	5.40±0.07 a, D	5.18±0.05 a, E	4.59±0.10 a, F	4.57±0.02 a, F	4.49±0.05 a, F	4.69 ±0.07 a, F
Sugar content	0.1% (w/v)	6.62±0.17 a, A	5.92±0.02 a, B	5.94±0.05 b, B	5.84±0.05 b, B, C	5.80±0.10 b, B, C	5.80±0.10 b, B, C	5.68±0.07 b, C, D	5.58±0.073 b, D	5.56±0.10 b, D	5.37±0.02 b, E	5.20±0.02 b, E
(g/100mL)	0.3% (w/v)	6.58±0.12 a, A	6.29±0.05 b, B	6.04±0.05 b, C	5.91±0.05 b, D, E	5.96±0.02 c, C, D	5.87±0.05 b, D, E	5.80±0.05 b, E, F	5.72±0.07 b, F, G	5.58±0.02 b, H	5.61±0.02 c, G, H	5.51±0.02 c, H
	0.5% (w/v)	6.63±0.15 a, A	6.58±0.07 c, A, B	6.43±0.10 c, B, C	6.29±0.05 c, C, D	6.18±0.05 d, D, E	6.17±0.02 c, D, E	6.08±0.10 c, E, F	6.03±0.07 c, E, F	6.01±0.10 c, E, F	5.94±0.10 d, F	5.75±0.07 d, G
	Control	4.89±0.01 c, A	4.67±0.02 c, B	4.50±0.05 b, C	4.39±0.01 c, C, D	4.27±0.03 c, C, D	4.12±0.02 b, C, D	3.99±0.01 b, D, E, F	3.89±0.01 a, D, E, F	3.83±0.01 a, E, F	3.73±0.02 a, E, F	3.66±0.01 a, F
pH	0.1% (w/v)	4.82±0.02 b, A	4.63±0.02 a, b, B	4.45±0.01 b, c, C	4.31±0.02 b, D	4.20±0.01 b, E	4.08±0.02 a, b F	3.97±0.01 b, G	3.91±0.03 a, b, H	3.88±0.02 b, I	3.78±0.03 b, J	3.75±0.02 b, J
	0.3% (w/v)	4.77±0.02 a, A	4.61±0.01 a, B	4.37±0.03 a, C	4.28±0.03 a, D	4.15±0.02 a, E	4.04±0.04 a, F	3.95±0.02 b, G	3.93±0.02 a, b, G	3.84±0.01 a, H	3.78±0.03 b, I	3.74±0.00 b, J
	0.5% (w/v)	4.74±0.02 a, A	4.64±0.02 b, c B	4.34±0.02 a, C	4.28±0.01 a, D	4.11±0.03 a, E	4.04±0.05 a, F	3.94±0.01 a, G	3.92±0.01 a, G	3.86±0.02 a, b, H	3.77±0.01 b, I	3.75±0.01 b, J

Mean values (n=3) in each column and row are followed by different normal and capital superscript letters, resepctively ($p \leq 0.05$)

Prince of Songkla University Pattani Campus

Table 11 The cell viability (log CFU/mL) of *A. aceti* TISTR 102, reducing sugar content (g/100mL) and pH values as culturing *A. aceti* TISTR 102 in coconut water with 50 % (v/v) banana juice medium at different concentrations of yeast extract

Time (hour)		**0**	**6**	**12**	**18**	**24**	**30**	**36**	**42**	**48**	**60**	**72**
Cell viability (log CFU/mL)	Control	6.86±0.01 a, A	7.61±0.06 a, B	8.14±0.04 a, C	8.35±0.02 b, D	8.38±0.06 a, D	8.52±0.05 b, E	8.48±0.01 a, b, E	8.50±0.02 a, E	8.46±0.01 a, E	8.37±0.03 a, D	8.30±0.04 a, D
	0.2% (w/v)	6.85±0.01 a, A	7.59±0.02 a, B	8.11±0.06 a, C	8.14±0.02 a, C	8.36±0.10 a, E	8.40±0.04 a ,b, E	8.41±0.04 a, E	8.43±0.01 a, E	8.42±0.00 a, E	8.39±0.03 a, b, E	8.25±0.03 a, D
	0.6% (w/v)	6.86±0.02 a, A	7.55±0.00 a, B	8.11±0.02 a, C	8.18±0.07 a, C	8.28±0.14 a, D	8.37±0.01 a, D, E	8.41±0.05 a, D, E	8.54±0.06 a, E	8.45±0.06 a, E	8.43±0.02 a, b, E, F, G	8.42±0.02 b, D, E
	1.0% (w/v)	6.86±0.05 a, A	7.53±0.04 a, B	8.06±0.04 a, C	8.17±0.04 a, D	8.34±0.02 a, E	8.36±0.05 a, E, F	8.48±0.06 a, G	8.46±0.09 a, F,G	8.45±0.03 a, E, F, G	8.45±0.00 b, E, F, G	8.46±0.03 b, F, G
Sugar content (g/100mL)	Control	7.90±0.07 a, A	7.60±0.05 a, B	7.41±0.07 a, C	7.39±0.06 a, C, D	7.26±0.05 a, D, E	7.12±0.05 a, E	6.96±0.07 a, F	6.84±0.05 a, F, G	6.75± 0.07 a, G, H	6.65±0.07 a, H	6.44±0.07 a, I
	0.2% (w/v)	8.21±0.07 b, A	7.69±0.02 a, B	7.59±0.02 a, B, C	7.50±0.05 a, C, D	7.39±0.05 a, D, E	7.31±0.02 b, E	7.31±0.07 b, E	7.34±0.07 b, E	7.07± 0.07 b, F	6.93±0.07 b, G	6.58±0.07 a, b, H
	0.6% (w/v)	8.35±0.07 b, c, A	7.98±0.10 b, B	7.93±0.07 b, B, C	7.86±0.07 b, B, C, D	7.86±0.12 b, B, C, D	7.78±0.05 c, C, D, E	7.69±0.07 c, D, E	7.62±0.07 c, E	7.29±0.10 c, F	7.07±0.07 b, G	6.79±0.07 b, c, H
	1.0% (w/v)	8.47±0.05 c, A	8.12±0.05 b, B	8.07±0.07 b, B	7.97±0.02 b, B, C	7.97±0.07 b, B, C	7.90±0.07 c, C	7.71±0.10 c, D	7.62±0.07 c, D	7.59± 0.02 d, D	7.31±0.07 c, E	7.19±0.10 c, E
pH	Control	4.84±0.0 a, A	4.66±0.01 a, B	4.53±0.01 a, C	4.49±0.01 a, D	4.43±0.01 a, E	4.34±0.02 a, F	4.23±0.03 a, G	4.12±0.02 a, H	4.04±0.02 a, I	3.90±0.01 a, J	3.82±0.01 a, K
	0.2% (w/v)	4.86±0.01 b, A	4.68±0.01 b, B	4.57±0.01 b, C	4.53±0.01 b, D	4.51±0.01 b, E	4.43±0.01 b, F	4.34±0.01 b, G	4.24±0.01 b, H	4.17±0.01 b, I	4.00±0.01 b, J	3.93±0.01 b, J
	0.6% (w/v)	4.87±0.0 b, A	4.73±0.01 c, B	4.64±0.02 c, C	4.57±0.01 c, D	4.58±0.01 c, E	4.53±0.01 c, F	4.47±0.02 c, G	4.38±0.03 c, G	4.29±0.02 c, H	4.11±0.02 c, I	4.01±0.01 c, J
	1.0% (w/v)	4.88±0.02 b, A	4.77±0.03 d, B	4.69±0.01 d, C	4.64±0.01 d, D	4.63±0.01 d, E	4.58±0.02 d, F	4.56±0.01 d, G	4.48±0.02 d, H	4.39±0.02 d, I	4.19±0.02 d, J	4.09±0.01 d, K

Mean values (n=3) in each column and row are followed by different normal and capital superscript letters, resepctively ($p \leq 0.05$)

Prince of Songkla University Pattani Campus

92

Table 12 The cell viability (log CFU/mL) of *A. aceti* TISTR 102, reducing sugar content and pH values as culturing *A. aceti* TISTR 102 in coconut water with 50 % (v/v) added banana juice medium at different shaking speeds

Time (hour)		**0**	**6**	**12**	**18**	**24**	**30**	**36**	**42**	**48**	**60**	**72**
Cell viability	120 rpm	6.72±0.04 a, A	7.56±0.03 a, B	7.76±0.18 a, C	8.21±0.13 a, D	8.46±0.05 a, E	8.57±0.02 a, E	8.53±0.03 a, E	8.55±0.07 a, E	8.50±0.08 a, E	8.50±0.05 a, E	8.49±0.04 a, E
(log CFU/mL)	150 rpm	6.73±0.08 a, A	7.61±0.08 a, B	8.06±0.05 b, C	8.45±0.03 b, D	8.56±0.02 b, E	8.69±0.05 b, E	8.67±0.04 b, E	8.67±0.02 b, E	8.66±0.06 b, E	8.59±0.04 b, E	8.59±0.08 a, E
Sugar content	120 rpm	7.57±0.15 a, A	7.34±0.02 a , B	7.27±0.02 a, B, C	7.26±0.10 b, B, C	7.17±0.07 b, C, D	7.07±0.02 b, D	6.84±0.05 a, E	6.74±0.05 a, E	6.70±0.05 a, E	6.48±0.07 a, F	6.24±0.07 a, G
(g/100mL)	150 rpm	7.53±0.05 a, A	4.29±0.10 a, B	7.15±0.05 a, C	6.94±0.05 a, D	6.79±0.07 a, E	6.62± 0.07 a, F, G	6.63±0.00 b, F	6.58±0.07 a, F, G	6.48±0.07 a, G	6.22±0.05 a, H	6.04±0.05 a, I
pH	120 rpm	4.91±0.01 a, A	4.74±0.00 b, B	4.58± 0.01 a, C	4.53±0.01 a, D	4.50±0.00 b, E	4.42±0.01 a, F	4.26±0.01 a, G	4.13±0.01 a, H	4.0±0.01 a, I	3.88±0.01 a, J	3.80±0.01 a, K
	150 rpm	4.92±0.00 a, A	4.73±0.01 a, B	4.56± 0.01 a, C	4.52± 0.01 a, D	4.49±0.01 a, E	4.39±0.01 a, F	4.25±0.01 a, G	4.13±0.01 a, H	4.05±0.01 a, I	3.91±0.01 b, J	8.59±0.01 b, K

Mean values (n=3) in each column and row are followed by different normal and capital superscript letters, resepctively ($p \leq 0.05$)

Prince of Songkla University Pattani Campus

Table 13 The moisture content (%), water activity (a_w) and cell viability of *A. aceti* TISTR 102 starter powder (log CFU/g) before and after drying as suspending into different sucrose concentrations

	Treatments	Before drying	After drying
Moisture content (%)	Control	32.86±0.37 ^{d}	8.27±0.08 ^{b}
	10% (w/v) sucrose	30.79±0.57 ^{c}	8.85±0.22 ^{a}
	20% (w/v) sucrose	29.70±0.35 ^{b}	9.18±0.41 ^{a}
	30% (w/v) sucrose	28.59±0.27 ^{a}	9.30±0.31 ^{a}
Water activity	Control	0.947±0.001 ^{d}	0.552±0.019 ^{a}
	10% (w/v) sucrose	0.935±0.002 ^{c}	0.563±0.017 ^{a}
	20% (w/v) sucrose	0.926±0.001 ^{b}	0.543±0.011 ^{a}
	30% (w/v) sucrose	0.917±0.002 ^{a}	0.545±0.004 ^{a}
Cell viability (log CFU/g)	Control	7.78±0.10 ^{d}	5.23±0.22 ^{a}
	10% (w/v) sucrose	7.34±0.14 ^{c}	5.25±0.08 ^{a}
	20% (w/v) sucrose	6.86±0.23 ^{b}	5.38±0.10 ^{a}
	30% (w/v) sucrose	6.46±0.14 ^{a}	5.28±0.05 ^{a}

Table 14 The moisture content (%), water activity (a_w) and cell viability of *A. aceti* TISTR 102 starter powder (log CFU/g) during storage time with and without silica gel

Storage days		0	10	20	30	40	50	60
Moisture content (%)	With silica gel	7.85±0.32 ^{A}	8.13±0.31 ^{A}	8.66±0.10 ^{B}	8.82±0.15 ^{B}	8.88±0.29 ^{B}	8.95±0.04 ^{B}	9.07± 0.12 ^{B}
	Without silica gel	7.73± 0.11 ^{A}	8.30±0.37 ^{B}	8.94±0.14 ^{C}	9.05±0.07 ^{C}	9.16±0.04 ^{C}	9.02±0.29 ^{C}	9.49±0.06 ^{D}
Water activity (a_w)	With silica gel	0.571±0.006 ^{A}	0.570±0.003 ^{A}	0.570±0.013 ^{A}	0.568±0.006 ^{A}	0.579±0.005 ^{A}	0.575±0.003 ^{A}	0.571±0.005 ^{A}
	Without silica gel	0.609±0.007 ^{A}	0.600±0.008 ^{A,B}	0.593±0.005 ^{B}	0.592±0.003 ^{B}	0.600±0.003 $^{A, B}$	0.597±0.002 ^{B}	0.599±0.001 ^{B}
Cell viability (log CFU /g)	With silica gel	5.89±0.05 ^{A}	5.89±0.30 ^{A}	5.89±0.19 ^{A}	5.74±0.15 ^{A}	5.70±0.15 ^{A}	5.65±0.05 ^{A}	4.96±0.02 ^{B}
	Without silica gel	5.91± 0.08 ^{A}	5.90±0.06 ^{A}	5.85±0.19 ^{A}	5.76±0.10 ^{A,B}	5.61±0.07 ^{B}	5.58±0.08 ^{B}	4.95±0.16 ^{C}

Mean values (n=3) in each column and row are followed by different normal and capital superscript letters, resepctively ($p \leq 0.05$)

94

Prince of Songkla University Pattani Campus

Table 15 The ethanol content (% v/v), sugar content (g/100mL), total soluble solid (^{o}Brix) and pH values as fermenting coconut water with different baker's yeast concentrations

	Time (Day)	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Ethanol	0.2% baker's yeast	0.00±0.00 $^{a, A}$	3.64±0.08 $^{a, B}$	7.76±0.20 $^{a, C}$	9.92±0.18 $^{a, D}$	9.82±0.26 $^{a, D}$	9.88±0.37 $^{a, D}$	9.70±0.25 $^{a, D}$	9.76±0.34 $^{a, D}$
content	0.4% baker's yeast	0.00±0.00 $^{a, A}$	6.18±0.22 $^{b, B}$	9.81±0.18 $^{b, C}$	9.92±0.18 $^{a, C}$	9.82±0.26 $^{a, C}$	9.82±0.26 $^{a, C}$	9.82±0.26 $^{a, C}$	9.8 ±0.35 $^{a, C}$
	0.2% baker's yeast	0.00±0.00 $^{a, A}$	7.57±0.24 $^{c, B}$	9.86±0.10 $^{b, C}$	9.86±0.10 $^{a, C}$	9.81±0.18 $^{a, C}$	9.81±0.^{10} $^{a, C}$	9.70±0.09 $^{a, C}$	9.76±0.16 $^{a, C}$
Sugar	0.2% baker's yeast	14.25±0.68 $^{a, A}$	7.78±0.23 $^{c, B}$	1.98±0.21 $^{b, C}$	0.34±0.04 $^{b, D}$	0.19±0.02 $^{b, D}$	0.09±0.02 $^{c, D}$	0.08±0.00 $^{a, D}$	0.06±0.01 $^{a, D}$
content	0.4% baker's yeast	14.25±0.68 $^{a, A}$	6.19±0.58 $^{b, B}$	0.13±0.01 $^{a, C}$	0.05±0.01 $^{a, C}$	0.05±0.01 $^{a, C}$	0.06±0.01 $^{b, C}$	0.05±0.02 $^{a, C}$	0.04±0.01 $^{a, C}$
	0.6% baker 's yeast	14.25±0.68 $^{a, A}$	2.70±0.15 $^{a, B}$	0.07±0.02 $^{a, C}$	0.06±0.00 $^{a, C}$	0.05±0.02 $^{a, C}$	0.03±0.05 $^{a, C}$	0.04±0.00 $^{a, C}$	0.04±0.00 $^{a, C}$
Total	0.2% baker's yeast	18.0±0.2 $^{a, A}$	12.9±0.2 $^{c, B}$	8.6±0.4 $^{b, C}$	6.1±0.2 $^{a, D}$	6.2±0.3 $^{a, D}$	6.3±0.2 $^{a, D}$	6.1±0.3 $^{a, D}$	6.1±0.2 $^{a, D}$
soluble	0.4% baker's yeast	18.0±0.2 $^{a, A}$	10.4±0.3 $^{b, B}$	6.1±0.2 $^{a, C}$	6.1±0.1 $^{a, C}$	6.3±0.2 $^{a, C}$	6.4±0.2 $^{a, C}$	6.2±0.2 $^{a, C}$	6.2±0.2 $^{a, C}$
Solid	0.6% baker's yeast	18.0±0.2 $^{a, A}$	9.2±0.4 $^{a, B}$	6.0±0.1 $^{a, D}$	6.2±0.1 $^{a, C, D}$	6.3±0.2 $^{a, C, D}$	6.4±0.2 $^{a, C, D}$	6.4±0.1 $^{a, C}$	6.3±0.2 $^{a, C, D}$
	0.2% baker's yeast	5.50±0.02 $^{a, A}$	4.32±0.01 $^{b, B}$	4.09±0.01 $^{a, E}$	4.05±0.01 $^{a, F}$	4.08±0.00 $^{a, E}$	4.13±0.01 $^{a, D}$	4.15±0.01 $^{a, C}$	4.16±0.01 $^{a, C}$
pH	0.4% baker's yeast	5.50±0.02 $^{a, A}$	4.20±0.02 $^{a, C}$	4.14±0.03 $^{b, D}$	4.16±0.02 $^{b, D}$	4.20±0.02 $^{b, C}$	4.25±0.02 $^{b, B}$	4.25±0.02 $^{b, B}$	4.28±0.03 $^{b, B}$
	0.6% baker's yeast	5.50±0.02 $^{a, A}$	4.19±0.02 $^{a, E}$	4.17±0.01 $^{b, F}$	4.20±0.01 $^{c, E}$	4.24±0.01 $^{c, D}$	4.28±0.01 $^{c, C}$	4.29±0.01 $^{c, B, C}$	4.31±0.02 $^{b, B}$

Mean values (n=3) in each column and row are followed by different normal and capital superscript letters, resepctively ($p \leq 0.05$)

Prince of Songkla University Pattani Campus

Table 16 The ethanol content (% v/v), sugar content (g/100mL), total soluble solid (^{o}Brix) and pH values as fermenting coconut water at different sugar contents

Time (Day)		0	1	2	3	4	5	6	7
Ethanol	12% (w/v) sugar	0.00±0.00 ^{A}	6.27±0.14 $^{a, B}$	6.38±0.11 $^{a, B, C}$	6.49±0.00 $^{a, C}$	6.51±0.09 $^{a, C}$	6.41±0.14 $^{a, B, C}$	6.43±0.12 $^{a, B, C}$	6.41±0.08 $^{a, B, C}$
content	16% (w/v) sugar	0.00±0.00 ^{A}	6.70±0.00 $^{b, B}$	9.61±0.17 $^{b, C}$	9.69±0.04 $^{b, C}$	9.70±0.09 $^{b, C}$	9.65 ± 0.09 $^{b, C}$	9.65 ± 0.09 $^{b, C}$	9.58 ± 0.08 $^{b, C}$
	20% (w/v) sugar	0.00±0.00 ^{A}	7.40±0.09 $^{c, B}$	10.78±0.05 $^{c, C}$	12.37±0.06 $^{c, D}$	12.73±0.06 $^{c, E}$	13.03±0.12 $^{c, F}$	12.87±0.06 $^{c, E, F}$	12.90±0.10 $^{c, E, F}$
Sugar	12% (w/v) sugar	12.55±0.71$^{a, A}$	0.44±0.00 $^{a, B}$	0.23±0.01 $^{a, B}$	0.17±0.01 $^{a, B}$	0.19±0.07 $^{a, B}$	0.17±0.03 $^{a, B}$	0.16±0.02 $^{a, B}$	0.22±0.03 $^{a, B}$
content	16% (w/v) sugar	16.11±0.46^{b,A}	5.11±0.11 $^{b, B}$	0.25±0.01 $^{a, C}$	0.20±0.01 $^{a, C}$	0.20±0.01 $^{a, C}$	0.20±0.05 $^{a, b, C}$	0.19±0.01 $^{b, C}$	0.23±0.03 $^{a, C}$
	20% (w/v) sugar	21.12±0.19$^{c, A}$	13.85±0.69$^{c, B}$	2.41±0.49 $^{b, C}$	1.12 ±0.34 $^{b, D}$	0.41±0.02 $^{b, D, E}$	0.30±0.01 $^{b, E}$	0.27±0.01 $^{c, E}$	0.35±0.03 $^{b, D, E}$
Total	12% (w/v) sugar	13.0±0.20 $^{a, A}$	5.07±0.06 $^{a, B}$	5.07±0.12 $^{a, B}$	5.10±0.10 $^{a, B}$	5.10±0.10 $^{a, B}$	5.08±0.10 $^{a, B}$	5.03±0.06 $^{a, B}$	5.00±0.10 $^{a, B}$
soluble	16% (w/v) sugar	17.17±0.21^{b,A}	9.60±0.10 $^{b, B}$	6.37±0.12 $^{b, C}$	6.43±0.06 $^{b, C}$	6.43±0.06 $^{b, C}$	6.43±0.06 $^{b, C}$	6.33±0.15 $^{b, C}$	6.33±0.12 $^{b, C}$
Solid	20% (w/v) sugar	21.13±0.12$^{c, A}$	15.10±0.17$^{c, B}$	10.30±0.10 $^{c, C}$	8.43±0.06 $^{c, D}$	8.00±0.10 $^{c, E}$	8.00±0.00 $^{c, E}$	7.92±0.10 $^{c, E}$	8.00±0.10 $^{c, E}$
	12% (w/v) sugar	5.50±0.01 $^{a, A}$	4.18±0.01 $^{b, E}$	4.12±0.01$^{c, F}$	4.13±0.01 $^{c, F}$	4.16±0.01 $^{c, E}$	4.22±0.03 $^{b, D}$	4.24±0.01 $^{c, C}$	4.28±0.01 $^{c, B}$
pH	16% (w/v) sugar	5.50±0.02 $^{a, A}$	4.04±0.01 $^{a, E}$	3.99±0.01$^{b, G}$	4.02±0.01 $^{b, F}$	4.07±0.02 $^{b, D}$	4.08±0.01 $^{a, D}$	4.12±0.01 $^{b, C}$	4.15±0.01 $^{b, B}$
	20% (w/v) sugar	5.50±0.02 $^{a, A}$	4.05±0.01 $^{a, D}$	3.96±0.01$^{a, F}$	3.93±0.01 $^{a, G}$	4.00±0.01 $^{a, E}$	4.05±0.01 $^{a, D}$	4.08±0.01 $^{a, C}$	4.10±0.01 $^{a, B}$

Mean values (n=3) in each column and row are followed by different normal and capital superscript letters, resepctively ($p \leq 0.05$)

Prince of Songkla University Pattani Campus

Table 17 The ethanol, acidity and pH changes during fermentation time with different *A. aceti* TISTR 102 starter powder concentrations

	Time	**0**	**3**	**6**	**9**	**12**	**15**	**18**	**21**	**24**	**27**	**30**
	0.5%	6.53± 0.00 a, A	6.70±0.25 a, A	5.76±0.09 a, B	4.51±0.28 a, C	3.00±0.07 a, D	0.99±0.13 a, E	0.00±0.00 a, F	0.00±0.00 F	0.00±0.00 F	0.00±0.00 F	0.00±0.00 F
Ethanol	1.0%	6.85±0.14 a, A	6.78±0.04 a, A	6.04±0.09 b, B	5.17±0.05 b, C	3.81±0.03 b, D	1.99±0.23 b, E	0.29±0.05 a, F	0.00±0.00 G	0.00±0.00 G	0.00±0.00 G	0.00±0.00 G
(% v/v)	1.5%	6.67±0.19 a, A	6.72±0.26 a, A	5.90±0.11 a, b, B	5.07±0.09 b, C	3.67±0.45 b, D	1.88±0.40 b, E	0.31±0.18 a, F	0.00±0.00 F	0.00±0.00 F	0.00±0.00 F	0.00±0.00 F
	2.0%	6.78±0.25 a, A	6.69±0.40 a, A	6.04±0.09 b, B	5.25±0.23 b, C	4.31±0.11 b, D	2.04±0.62 b, E	0.37±0.23 a, F	0.00±0.00 F	0.00±0.00 F	0.00±0.00 F	0.00±0.00 F
	0.5%	0.24±0.00 a, A	0.24±0.02 a, A	0.87±0.05 a, B	1.94±0.05 b, C	3.25±0.07 c, D	4.87±0.14 a, E	6.27±0.02 b, H	6.16±0.00 a, H	6.01±0.21 a, G, H	5.85±0.24 a, F, G	5.69±0.11 a, F
Acidity	1.0%	0.24±0.00 a, A	0.26±0.00 a, A	0.90±0.01 a, B	1.85±0.05 a, b, C	3.02±0.06 b, D	4.65±0.04 a, E	6.04±0.14 a, b, F	6.43±0.02 a, F	6.27±0.08 a, F	5.95±0.23 a, F	4.82±0.66 a, E
(g/100mL)	1.5%	0.24±0.00 a, A	0.26±0.01 a, A	0.77±0.25 a, A	1.83±0.02 a, b, B	2.91±0.08 a, b, C	4.52±0.27 a, D	5.89±0.27 a, b, F, G	6.32±0.31 a, G	6.16±0.33 a, G	5.49±0.39 a, E, F	4.97±0.49 a, D, E
	2.0%	0.24±0.00 a, A	0.27±0.01 a, A	0.80±0.00 a, A	1.72±0.08 b, D, B	2.77±0.02 a, D, C	4.38±0.23 a, D	5.73±0.12 a, F	6.23±0.23 a, F	6.21±0.41 a, F	5.74±0.27 a, F	5.06±0.66 a, D
	0.5%	4.15±0.01 a,B	4.34±0.12[a,] A	3.96±0.03 a, C	3.59±0.01 a, D	3.43±0.002 a, E	3.23±0.00 a, F	3.03±0.01 a, G	3.21±0.00 a, F	3.20±0.06 [a, F]	3.22±0.05 a, F	3.17±0.01 a, F
pH	1.0%	4.10±0.04 a,B	4.31±0.01[a,] A	3.96±0.04 a, C	3.65±0.05 a, b, D	3.51±0.01 a, b, E	3.26±0.01 b, F	3.04± 0.00 a, b, G	3.21±0.01 a, F	3.22±0.01 a, F	3.21±0.01 a, F	3.23±0.04 a, F
	1.5%	4.10±0.05 a,B	4.33±0.01 a, b, A	3.99±0.04 a, C	3.64±0.01 a, D	3.52±0.04 b, E	3.31±0.01 c, F	3.07±0.02 b, B, H	3.22±0.01 a, G	3.25±0.01 a, F, G	3.27±0.03 a, F, G	3.26±0.04 a, F, G
	2.0%	4.10±0.01 a,B	4.50±0.05[b,] A	4.11±0.01[b,] B	3.71±0.04 b,C	3.61±0.04[c,] D	3.36±0.01[d,E]	3.14±0.01[c,] H	3.30±0.01 b, E, F	3.25±0.00 a, F. G	3.26±0.00 a, F, G	3.22±0.02 a, G

Mean values (n=3) in each column and row are followed by different normal and capital superscript letters, resepctively ($p \leq 0.05$)

Prince of Songkla University Pattani Campus

VITAE

Name Thao Ngan Ngoc Trinh

Student ID 5620320403

Educational Attainment

Degree	Name of Institution	Year of Graduation
Barchelor in Food Science and Technology	Nong Lam University, Vietnam	2013

Scholarship Awards during Enrolment

SAT-ASEAN Scholarship for International Student (2013) from Faculty of Science and Technology, Prince of Songkla University, Pattani Campus, Thailand

List of Publication and Conference

Trinh, N.T.N., Masniyom, P. and Maneesri, J. 2015. Preparation of Vinegar from Coconut Water Using baker's yeast and *Acetobacter aceti* TISTR 102 starter powder. In the 6th International Conference on Fermentation Technology for Value Added Agricultural Products, Khon Kaen, 29-31 July, 2015.

Trinh, N.T.N., Masniyom, P. and Maneesri, J. 2015. Optimization of culture conditions for *Acetobacter aceti* TISTR 102 in coconut water with supplementary banana juice. International Journal Food Journal. (Submitted)

Prince of Songkla University Pattani Campus