OPTIMIZATION OF BEER PRODUCTION FROM RICE MALT BASED USING RESPONSE SURFACE METHODOLOGY

ARTIT KONGKAEW

A Thesis Submitted in Partial Fulfillment of the Requirements for the

Degree of Master of Science in Biotechnology

Suranaree University of Technology

Academic Year 2010

การหาสภาวะที่เหมาะสมในการผลิตเบียร์จากข้าวเป็นส่วนประกอบหลักโดยวิธีพื้นที่ผิวตอบสนอง

นายอาทิตย์ กองแก้ว

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาเทคโนโลยีชีวภาพ
มหาวิทยาลัยเทคโนโลยีสุรนารี
ปีการศึกษา 2553

OPTIMIZATION OF BEER PRODUCTION FROM RICE MALT BASED USING RESPONSE SURFACE METHODOLOGY

Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for a Master's Degree.

Thesis Examining Committee

_____
(Assoc. Prof. Dr. Montarop Yamabhai)
Chairperson

_____
(Asst. Prof. Dr. Chokchai Wanapu)
Member (Thesis Advisor)

_____
(Assoc. Prof. Dr. Neung Teaumroong)
Member (Thesis Co-Advisor)

_____
(Dr. Ulaiwan Usansa)
Member

มหาวิทยาลัยเทคโนโลยีสุรนารี

_____ _____
(Prof. Dr. Sukit Limpijumnong) (Asst. Prof. Dr. Suwayd Ningsanond)
Vice Rector for Academic Affairs Dean of Institute of Science

อาทิตย์ กองแก้ว : การหาสภาวะที่เหมาะสมในการผลิตเบียร์จากข้าวเป็นส่วนประกอบหลักโดยวิธีพื้นที่ผิวตอบสนอง (OPTIMIZATION OF BEER PRODUCTION FROM RICE MALT BASED USING RESPONSE SURFACE METHODOLOGY)
อาจารย์ที่ปรึกษา : ผู้ช่วยศาสตราจารย์ ดร. โชคชัย วนภู, 115 หน้า.

การวิจัยนี้ได้ทำการศึกษา และผลิตเบียร์โดยใช้ข้าวเป็นส่วนประกอบหลัก มีวัตถุประสงค์เพื่อลดต้นทุนการผลิตเบียร์ และเพิ่มมูลค่าของข้าวไทย ข้าวที่ใช้ในการศึกษานี้คือ ข้าวพันธุ์ผสม CP13 ซึ่งข้าวพันธุ์ดังกล่าวได้นำมาทำมอลต์โดยการทำให้งอกที่ 1 3 5 7 และ 9 วัน จากนั้นทำการหาค่าคุณภาพของมอลต์ข้าวที่ได้ พบว่า ค่า extract content และค่า Free amino nitrogen (FAN) ของมอลต์ข้าวจะเพิ่มขึ้นตั้งแต่วันที่ 3 ถึงวันที่ 9 ของระยะเวลาในการงอก โดยมอลต์ข้าววันที่ 9 ให้ค่า extract content สูงสุดที่ 46% อย่างไรก็ตาม ค่า extract content ของมอลต์ข้าวน้อยกว่าค่า extract content ของมอลต์บาร์เลย์ที่ใช้ผลิตเบียร์โดยทั่วไปที่ 80% สำหรับมอลต์ข้าววันที่ 9 ให้ค่า FAN สูงสุดที่ 145 มิลลิกรัมต่อมอลต์ 100 กรัม ซึ่งไม่แตกต่างอย่างมีนัยสำคัญทางสถิติกับ FAN ที่พบในมอลต์บาร์เลย์ที่ 150 มิลลิกรัมต่อมอลต์ 100 กรัม ค่า malting loss ของมอลต์ข้าวในวันที่ 7 และ 9 ของการงอกมีค่ามากกว่า 20% ซึ่งไม่เหมาะสมกับการผลิตมอลต์สำหรับทำเบียร์ เนื่องจากจะทำให้ต้นทุนการผลิตเบียร์ค่อนข้างสูง การศึกษาผลของเอนไซม์สองชนิด ได้แก่ เอนไซม์แอลฟา- อะไมเลส ซึ่งเป็นเอนไซม์ที่ทนความร้อนได้สูง เอนไซม์โปรติเอสจากแบคทีเรีย และมอลต์บาร์เลย์เพื่อใช้ในการเพิ่มคุณสมบัติของน้ำเวิร์ท โดยใช้วิธีพื้นที่ผิวตอบสนอง (RSM) และวิเคราะห์ความเหมาะสมของแบบจำลองโดยใช้ค่า *p*-value ผลการศึกษาพบว่า เวลาในการงอก เอนไซม์แอลฟา-อะไมเลส และมอลต์บาร์เลย์ มีผลต่อค่า extract content ผลผลิตของน้ำเวิร์ท และปริมาณ fermentable sugar ในขณะที่การงอกของข้าว เอนไซม์โปรติเอส มีผลต่อค่า FAN ในน้ำเวิร์ท อย่างไรก็ตาม เอนไซม์โปรติเอสที่มีอยู่ในมอลต์ข้าวมีผลต่อค่า FAN มากกว่าเอนไซม์โปรติเอสที่เติมเข้าไป นอกจากนี้ยังได้มีการการวิเคราะห์สภาวะ และปริมาณเชิงตัวเลขที่เหมาะสมต่อกระบวนการ mashing บนพื้นฐานของเวลาในการงอก และปริมาณมอลต์ข้าว

การผลิตเบียร์ระดับกึ่งอุตสาหกรรม พบว่า ปริมาณ fermentable sugar ทั้งหมดของน้ำเวิร์ทของข้าวที่งอก 5 วัน และข้าวมอลต์ 50% ไม่มีความแตกต่างกับเวิร์ทมาตรฐานที่ความเข้มข้นเท่ากัน อย่างไรก็ตาม ปริมาณน้ำตาลมอลโตสในน้ำเวิร์ทมาตรฐานมีมากกว่าน้ำเวิร์ทจากการทดลองประมาณ 3 เท่า ซึ่งในขณะที่ปริมาณน้ำตาลกลูโคสของน้ำเวิร์ทจากการทดลองค่อนข้างสูง คือประมาณ 33% ของปริมาณ fermentable sugar ทั้งหมด ส่วนเวลาในการงอกของมอลต์ข้าว และมอลต์บาร์เลย์สามารถเพิ่มการนำไปใช้ของ reducing sugar และ FAN ของยีสต์น้ำเวิร์ท อย่างไรก็

ตามการใช้ reducing sugar ของยีสต์สูงสุดคือ 70% ซึ่งน้อยกว่าค่ามาตรฐานที่ 80% เนื่องมาจากพบปริมาณน้ำตาล maltotriose หลงเหลืออยู่ และอาจเป็นสาเหตุทำให้ปริมาณเอทานอลในตัวอย่างเบียร์ค่อนข้างต่ำ ปริมาณข้าวยังมีผลทำให้สีของตัวอย่างเบียร์เข้มขึ้นเนื่องมาจากกระบวนการ Maillard reaction ของเปลือกข้าวในระหว่างกระบวนการ mashing และ wort boiling

ปริมาณ isoamyl alcohol ในตัวอย่างเบียร์ที่ทำการทดลองอยู่ในช่วง 570 – 700 ppm ซึ่งมีมากกว่าปริมาณ isoamyl alcohol ในเบียร์มาตรฐานประมาณ 10 เท่า ปริมาณข้าว และเวลาในการงอกของข้าวมีผลทำให้เกิดการสร้างสารประกอบกลุ่ม ester เพิ่มขึ้น ได้แก่ isoamyl acetate ethyl octanoate และ ethyl decanoate เนื่องจากในข้าวมีสารประกอบกลุ่ม volatile fatty acid ค่อนข้างสูง ซึ่งเป็นสารตั้งต้นในการสังเคราะห์สารประกอบกลุ่ม ester ในการทดสอบทางประสาทสัมผัสของเบียร์โดยใช้ข้าวเป็นส่วนประกอบหลัก พบว่า เวลาในการงอกของข้าวไม่มีผลเมื่อมีการใช้มอลต์บาร์เลย์ที่ 50% ในทางกลับกันเวลาในการงอกของข้าวมีผลทางประสาทสัมผัสเมื่อมีการใช้ข้าวมอลต์มากกว่าหรือเท่ากับ 70% ผู้ทำการทดสอบได้ตัดสินตัวอย่างเบียร์ที่มีส่วนผสมของมอลต์ข้าว 50% อยู่ในเกณฑ์ดี ในขณะที่ตัวอย่างเบียร์ที่มีส่วนผสมของมอลต์ข้าว 70% อยู่ในเกณฑ์ปกติ และสามารถดื่มได้

มหาวิทยาลัยเทคโนโลยีสุรนารี

สาขาวิชาเทคโนโลยีชีวภาพ ลายมือชื่อนักศึกษา_____

ปีการศึกษา 2553 ลายมือชื่ออาจารย์ที่ปรึกษา_____

ARTIT KONGKAEW: OPTIMIZATION OF BEER PRODUCTION FROM RICE MALT BASED USING RESPONSE SURFACE METHODOLOGY. THESIS ADVISOR ASST. PROF. CHOCKCHAI WANAPU, Ph.D., 115 pp.

BEER PRODUCTION/ RICE MALT/ RESPONSE SURFACE METHODOLOGY

In order to reduce the cost of beer production, using rice as major ingredient in brewing process was investigated and established. Productions of rice malt (Hybrid Rice CP13) in 1st, 3rd, 5th, 7th, and 9th days of germination was carried out. Extract content and FAN constantly increased during the 3rd to 9th days of germination. The maximum extract content presented in 9th days of germination, but the extract content of rice not reached the typical brewing malt (80%). On the 9th day of germinated malt, it provided FAN for 145 mg/100 g malt, which was insignificant difference with FAN in malt barley (150 mg/100 g malt). On the other hand, the rice malt from 7th and 9th days of germination was negligible because of more than 20% malting loss. Commercial enzymes including heat stable α-amylase, bacterial protease, and malt barley were supplemented for improving the qualities of wort for brewing before optimization using RSM technique. The suitability of this model was analyzed and expressed as *p*-value. Germination time of rice, commercial α-amylase, and barley addition affected on extract content, yield, and fermentable sugar of wort. Whereas, germination time of rice and bacterial protease affected on FAN in wort. However, protease that generated in germinated malt showed higher impact on FAN than commercial enzyme addition. The numerical appropriate conditions for mashing condition based on germination time and rice malt ratios were determined.

Fifty-liters of beer productions of each treatment was done. Total fermentable sugars of wort from malt obtained at 5th day of germination at 50% were insignificant difference with standard wort at the same concentration. However, maltose concentration in standard wort was higher approximately 3 times than experimental wort, whereas amount of glucose from experimental wort was high about 33% of total fermentable sugar. Germination time of rice malt and barley malt addition improved both reducing sugar and FAN consumption. Nevertheless, the highest utilization of reducing sugar from the experiment was 70%, which was lower than reducing sugar from malt barley wort at more than 80% utilization. The lack of maltotriose consumption occurred in every treatments, which might be the main cause of low ethanol production. Rice malt ratio influenced on the color of beer due to the Maillard reaction of rice husk during mashing and wort boiling.

Isoamyl alcohol in experimental beer was 570 – 700 ppm, which 10 times higher than its beer standard. The rice malt ratio and germination time improved the formation of estery compounds in final beer including isoamyl acetate, ethyl octanoate, and ethyl decanoate. This might be due to the high level of volatile fatty acid, which is the precursor of estery compounds synthesis. Qualities of rice malt had no effect on sensory score when the 50% of barley malt was supplemented. Conversely, the ratio of rice malt to 70% influenced the qualities of rice malt. Twelve panelists judged the beer from 50% of rice malt as good, while beer from 70% of rice malt was judged as normal and drinkable.

School of Biotechnology Student's Signature_____

Academic Year 2010 Advisor's Signature_____

ACKNOWLEDGEMENTS

I would like to thank Suranaree University of Technology for financial and laboratory facilities supports during my study.

I am deeply grateful to my thesis advisor, Asst. Prof. Dr. Chokchai Wanapu and thesis co-advisor Assoc. Prof. Dr. Neung Teaumroong for guidance, valuable advices, encouragement, and other supports during this my work. I wish to express my gratitude to the dissertation committee, Dr. Ulaiwan Usansa for her comments and advices on my work. She always spent a lot of her time to help me solving some research problem.

I am very grateful to thank all staffs at the Biotechnology and Food Technology laboratories for their suggestion, and support of chemical reagents and instruments. I wish to thank P'Wan, P'Nut, P'Dang, P'Koy, P'Rong, P'Ngor, P'Guy, LiLi, Doy and Bow for their kind suggestion and friendship. I wish to thank Fon and P'Nok for advising and helping me whenever I needed.

Most of all, I would like to express my deepest gratitude to my parents and my sister who have continuously provided me with unconditional love and support.

Artit Kongkaew

CONTENTS

Page

CONTENTS (Continued)

Page

CONTENTS (Continued)

Page

CONTENTS (Continued)

Page

CONTENTS (Continued)

Page

LIST OF TABLES

Table **Page**

LIST OF TABLES (Continued)

Table **Page**

มหาวิทยาลัยเทคโนโลยีสุรนารี

LIST OF FIGURES

LIST OF FIGURES (Continued)

Figure **Page**

LIST OF FIGURES (Continued)

Figure **Page**

มหาวิทยาลัยเทคโนโลยีสุรนารี

LIST OF ABBREVIATIONS

AAT	=	Alcohol acetyl transferase
ANOVA	=	Analysis of varience
AOAC	=	Association of Official Analytical Chemists
ATP	=	Adenosine triphosphate
BU	=	Bitterness Unit
CRD	=	Completely Randomized Design
C.V.	=	Coefficient of variation
°C	=	Degree Celsius
DI	=	Deionize
DMRT	=	Duncan's Multiple Range Test
DMS	=	Dimethyl Sulfide
DNS	=	Dinitrosalicylic acid
DW	=	Dry weight
EBC	=	European Brewery Convention
eV	=	Electron volt
FAN	=	Free amino nitrogen
°F	=	Degree Fahrenheit
g	=	gram

มหาวิทยาลัยเทคโนโลยีสุรนารี

GA	=	Gibberellic acid
GC-MS	=	Gas chromatography coupled with mass spectroscopy

LIST OF ABBREVIATIONS (Continued)

g/L	=	Gram per liter
h	=	Hour
HPLC	=	High performance liquid chromatography
K^+	=	Potassium ion
kg	=	Kilogram
KNU	=	Kilo novo unit
L	=	Liter
M	=	Molar
mg	=	milligram
mL	=	milliliter
mm	=	millimeter
mM	=	millimolar
min	=	minute
nm	=	nanometer
No.	=	Number
°P	=	degree plato
PDMS	=	Polydimethylsiloxane
PSI	=	Pound square per inch
%	=	Percent
RI	=	Reflexive index

มหาวิทยาลัยเทคโนโลยีสุรนารี

RH = Relative humidity

LIST OF ABBREVIATIONS (Continued)

RHA = Rice hull ash

RO = Reverse osmosis

rpm = Round per minute

RSM = Response surface methodology

R^2 = R-square

RVA = Rapid visco analyser

SD = Standard deviation

SMM = S-methyl methionine

SPME = Solid phase microextraction

SPSS = Statistical Package for the Social Sciences

TUM = Technical University of Munich

μg = Microgram

μm = Micrometer

U.S.A. = United States of America

VDKs = Vicinal diketones

v/v = volume by volume

w/v = weight by volume

w/w = weight by weight

CHAPTER I

INTRODUCTION

1.1 Significant of research

Beer is the most popular alcoholic beverage achieved from the fermentation by yeast, which mostly uses malted barley as a starch source. However, barley does not grow in the tropical area, such as South-East Asia. Thus, the barley must be imported to Thailand.

Rice (*Oryza sativa*) is the most significant crops in the world as well as wheat and corn, which have been cultivated in over 100 countries around the world. Rice is the essential food for a half of the world population. Total production of rice paddy area is about 154 million hectares, and annual production of rice is about 731 million tons (Zhao *et al.*, 2010). However, rice is impractical for brewing because it contains very high starch content (85-95%), that swells greatly and gelatinizes at temperature 70-85°C due to the presence of very small starch granule. Furthermore, protein content is quiet low (5-8%), which not enough for yeast metabolism (Kanze, 2004), as well as illustrates the low foam stability and quality. Also, another cereal for example wheat, sorghum, and buckwheat besides rice have been studied the possibility for beer production and generated the novel beer approach (Agu and Palmer, 1998; Igyor *et al.*, 2001; Moonjai, 2005; Odibo *et al.*, 2002; Salubchua *et al.*, 2005; Wijngaard and

Arendt, 2006). Nowadays, the brewing industries become competitive. Manufacturers have tried to improve their beer qualities and reduce the raw materials cost. The utilization of rice for adjunct has been done in the brewing industry in order to reduce the costs of production. The application of rice should be used as the adjunct in brewing flavor such as neutral aroma, and taste (Goldammer, 2000). In addition, rice hull ash (RHA) is the effective filter aids for beer filtration. The results showed clarity, brightness and sensorial characteristics of beer, which were comparable to obtain with traditional filter aids (Villar et al, 2004).

Response surface methodology (RSM) is a collection of statistical and mathematical techniques, which is useful for development, improvement and optimization of the process. This method was established by Box and Wilson in 1951 (Myers and Montgomery, 2002). Equations were used to describe how the test variables affect the response, determine the interrelationship among the test variables, and describe the combined affect of all test variables in the responses (Giovanni, 1983).

Thailand is one of the largest rice exporters in the world. Thus, rice might be an interesting starch source for the new beer style production. The aims of this research is optimize the used of commercial enzymes with variation of barley malt and rice malt addition. Also, test for qualities of beer using rice malt as the major ingredient starch source for applying and reducing the cost of beer production. The designed all variable parameters were done by using RSM.

1.2 Research objectives

This research aims to develop and optimize the brewing process, which used rice as a major starch source in order to increase the value of rice, and reduce the cost of brewing production, with sub-objectives as follow;

1. To generate and determine the property of malt from rice
2. To generate the suitable mashing model to produce wort from rice malt as the major ingredient using RSM technique
3. To study the fermentation profile of rice beer using bottom fermenting yeast
4. To produce beer from rice as a major ingredient in 50-liters brewing scale and analyze the beer qualities

1.3 Research hypothesis

Malt from rice has efficiency been used for brewing as a source of carbohydrate. The modification and optimization of brewing process by the commercial enzymes and barley malt can improve the quality of rice beer, and reduce the cost of beer production.

1.4 Scope and limitations of the study

Firstly, the compositions of rice were determined in term of nutrition and physical properties including moisture content, protein content, fat content, ash content, and available carbohydrate as well as gelatinization temperature, and

percentage of germination. Thus, rice malt was produced and its qualities were analyzed. Small scale mashing and fermentation was done before a pilot scale of 50 liters production of beer. Small scale mashing method including mashing time, and commercial enzymes addition were optimized by using RSM. The suitable conditions were selected to study the fermentation kinetics such as ethanol production, and sugar utilization in 5-liters fermenter. The optimum condition from laboratory scale brewing was obtained in the 50-liters scale brewing. Finally, physical, chemical and sensory analyses of final beer were determined.

1.5 Expected result

The expected of result from this study is to obtain the new style beer product from rice, which is appropriate and acceptable qualities.

มหาวิทยาลัยเทคโนโลยีสุรนารี

CHAPTER II

LITERATURE REVIEWS

2.1 Definition of beer

Beer is the oldest and most popular alcoholic beverage in the world. It is produced from the fermentation of sugars, which derived from starch-based materials. The most common material is barley malt, and seasons with bitter taste hops. However wheat, corn, sorghum, and rice are also widely used in conjunction with barley (wikipedia, 2008).

มหาวิทยาลัยเทคโนโลยีสุรนารี

2.2 Basic ingredients of beer

The basic ingredients of beer are water, fermentable starch source (widely used as barley malt), yeast, and hops. A mixture of starch sources may be used as the secondary starch source such as corn, rice, and sugar often being termed as an adjunct especially used as a lower cost substitute for barley malt.

2.2.1 Starch source

The starch source provides the fermentable matter in beer and is the key of the quality of beer. Barley (*Hordeum vulgare*) is the most common starch source

used in beer. It is a cereal grasses, which has the flowers in dense spikes with long awns, and three spikelets at each joint of the “rachis”. Before barley is used in the brewery, they must be converted starch in endosperm to fermentable sugar known as malt.

2.2.1.1 Rice

Rice (*Oryza sativa* L.) is a cereal foodstuff, which forms an important part of the diet of a great population worldwide for at least 5,000 years. It is native to tropical and subtropical southern Asia, and southeastern Africa. Rice is widely grown throughout the world. Total rice production for 2004 is placed approximately 600 million metric tons per year. Thailand is the world class rice exporter, which produces more than 30 million metric tons of rice per year (Thai Rice Exporters Association, 2010) and highly diversity of rice variety approximately 85 cultivars have been promoted for plantation.

Structure of rice kernel is illustrated in figure 2.1. Rice consists of cover grain in which the kernel (caryopsis), which is encased in a protective hull. The hull has two-piece structures, which tightly encompasses the kernel for protect and the barrier to change in moisture content rapidly (Hettiarachchy *et al*., 2000). After the hull is removed, the caryopsis is known as brown rice which approximately 93% endosperm is situated. The major constituent of the endosperm is starch and the outer most layer of endosperm called aleurone layer. The area between hull and endosperm compose of three layers: pericarp, tegmen (seed coat), and nucellus account for approximately 3% of brown rice weight. Chemical compositions of brown and milled rice were shown in table 2.1.

Rice is currently the second most widely used as adjunct material in United State less than corn grits (Coors, 1976). The quality of rice for brewing can be judged by several factors including cleanliness, particle size, gelatinization temperature, mash viscosity, mash aroma, and protein content. Short

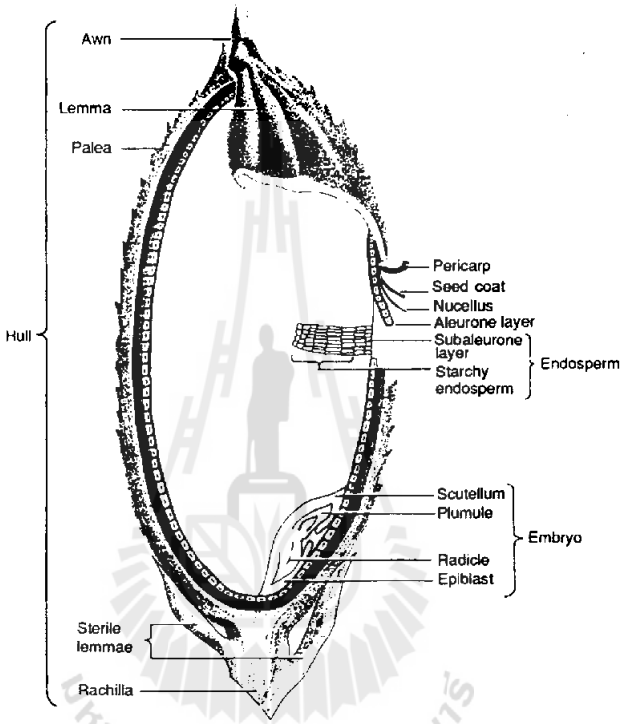


Figure 2.1 Structure of rice kernel

Source: www.fao.org

grain rice is preferred because of the low viscosity problem. However, rice has relatively high gelatinization temperature (70 - 85 °C) due to the presence of very small starch granule (2-10 μm) (Pierce, 1987; Lindeboom *et al.*, 2004). Rancidity of rice grain dues to the unfavorable storage condition such as high temperature and humidity, high lipid content of rice could be cause of the reduction of ester formation during the fermentation. (Anderson and Kirsop, 1974; Ayraapaa and Lindstrom,

1973). Furthermore, rice provides a low foam quality, stability and free amino nitrogen (FAN), which cause of the level of proteins and enzymes.

Table 2.1 Chemicals composition of brown and milled rice.

Nutrient	Brown rice	Milled rice
Starch (%)	57.0	67.0
Crude protein (%)	7.2	5.8
Crude fat (%)	1.9	0.3
Crude fiber (%)	0.7	0.3
Crude ash (%)	1.0	0.5
Niacin (μg/g)	43.0	18.0
Tocopherol(μg/g)	17.0	Trace
Thiamine (μg/g)	4.4	0.7
Riboflavin (μg/g)	0.9	0.4

Source: Adapted from Huey *et al*. (1988).

2.2.1.2 Barley

Barley (*Hordeum vulgare*) has been associated with the earliest beginnings of agriculture. Cultivated barley is must likely originated from wild progenitor 35,000 – 40,000 years ago (Hockett, 2000). Thus, barley is a cereal grains, which is applied many food application including beer and distilled beverage production, as a component of various health food also serving as a major of animal

fodder. Thus, barley can be divided depending on the arrangement of the corns on the ear axis as “two rows” and “multirows” barleys.

A structure of barley kernel in longitudinal cross-section is illustrated in figure 2.2, which consists of 3 main parts: germ region, endosperm, and grain covering. The germ region contains embryo with the growth for acrospires and rootlets. The endosperm contains starch granules in large and small size. The small starch granules with a diameter of 3 – 5 μm represent 70 – 95% of the starch granules in endosperm display major influence on the malting properties, and qualities of malt production (Kanze, 2004). The endosperm is surrounded by aleurone layer, the most important starting point for enzyme production during malting.

The grains covering consist of three difference layers. The inner most covering is the aleurone layer, following seed coat or testa and pericarp, and fruit coat. The outermost is “husk” that mainly consists of cellulose which can act adversely on the qualities of beer including polyphenol and bitter substances.

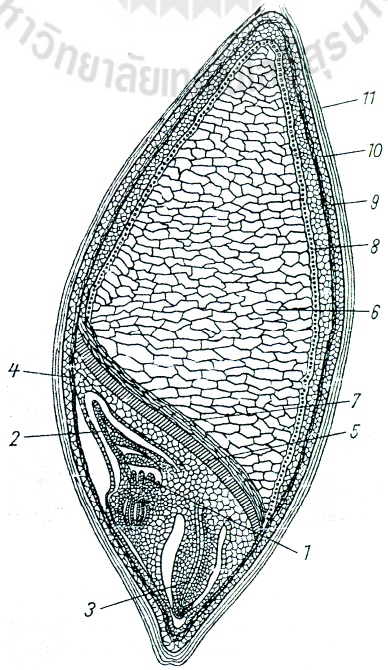


Figure 2.2 CA barley kernel in longitudinal cross-section (1) rudimentary stem, (2) rudimentary acrospire, (3) rudimentary rootlets, (4) scutellum, (5) epithelium layer, (6) endosperm, (7) empty cells, (8) aleurone layer, (9) testa (10) pericarp, and (11) husks

2.2.2 Water

Water is the quantitatively raw material for beer production, which always comes from a local source. Supply and preparation of water are largely important to the brewer because the water quality has an effect on the quality of beer.

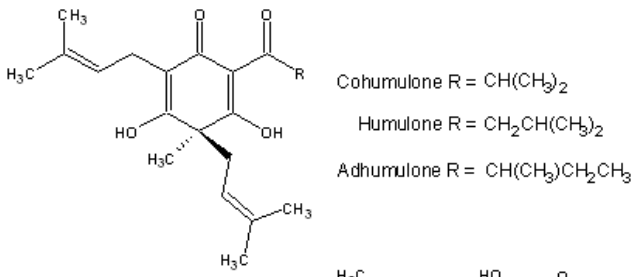
The ions present in water are significant, and influence on beer flavor, either beneficially or adversely by both direct and indirect effects, especially during wort production, wort boiling, and fermentation. The taste sensation has a direct effect on taste receptor. For example, Na^{+} ion and K^{+} ion can contribute a salty taste at concentration of 150 to 200 mg/L, and greater thanthat of 500 mg/L, respectively as well as Mg^{2+} ion can contribute a bitter, and sour flavor in beer, which above 70 mg/L (Taylor, 1981). Indirect effects of ion on beer flavor for instance yeast metabolism, which requires the appropriate concentration of elemental for accelerated growth, increased biomass yield, and enhance ethanol production. Furthermore, Ca^{2+} ion can directly stimulate amylopectic, activate proteolytic enzyme during wort production, and reduce color formation (Briggs *et al.*, 1981; Comrie, 1967; Taylor, 1990).

2.2.3 Hops

The hops are the female flowers of the hop plant (*Humulus lupulus L.*). A strong climbing plant is mainly used as flavour and stability agents in beer, others

beverage, and herbal medicine. The hops contribute floral, citrus, herbal aromas, and flavors to beer product. Particularly, soft resin of the hops contains α-acid and β-acid (figure 2.3), which are the primary bittering compounds (Rourke, 2003). There are three major forms of α-acid, and three analogous forms of β-acid. The α-acids are cohumulone, humulone, and adhumulone, whereas as the β-acids are colupulone, lupulone, and adlupulone (Robert and Wilson, 2005). The proportions of these compounds are remarkably consistent on hops varieties affecting on beer flavor and taste.

Several authors have suggested that the effect of antibiotic and positive antibacterial in hops due to the rich sources of flavones glycosides, especially xanthohumol, and others prenylated chalcones, which received much attention as cancer chemopreventive agents (Stevens and Page, 2004; Larsona *et al*., 1996; Natarajana *et al*., 2008). Furthermore, rho-α-acids from hops extract illustrated the anti-inflammatory activity and reduction of cardiovascular disease (Figarda *et al*., 2008; Hall *et al*., 2008).



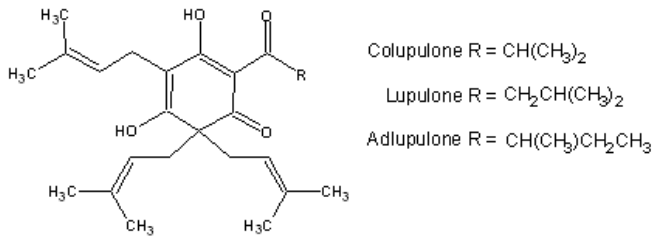


Figure 2.3 Chemical structures of the hop α-acids and β-acids.

Source: www.botanix.co.uk

2.2.4 Yeast

Yeast is the microorganism that responses for the fermentation in beer. It metabolizes sugar that extracted from grains to produce alcohol, and carbon dioxide also change wort into beer. In addition, yeast influences on the character, and flavors. Beer quality is strongly influence by the biochemical performance of yeast during fermentation. The ability of yeast to separate from beer at the required time, utilize sugar quickly and efficiently, high yield of ethanol, and balance of flavor compounds are the appropriate yeast for brewing (Powell *et al*., 2003). There are two types of beer yeast including ale yeast or top-fermenting yeast as *Saccharomyces cerevisiae*, and lager yeast or bottom-fermenting yeast as *S. uvarum* known as *S. carlsbergensis*.

2.2.4.1 Top-fermenting yeast

The ale yeast strains typically used at temperature ranging from 10 to 25°C. It is generally regarded as top-fermenting yeasts since they rise to the surface during fermentation, due to yeast flocculation, entrap CO_2, creating a very thick, and rich yeast head. Fermentation by the ale yeasts at rather warm temperatures, which produces a beer high in esters that regard as a character of ale beers. Top-

fermenting yeasts are used for brewing ales, porters, stouts, Altbier, Kölsch, and wheat beers.

2.2.4.2 Bottom-Fermenting yeast

The lager yeast strains typically be used at temperature ranging from 7 to 15°C. At these temperatures, lager yeasts grow slowly than ale yeasts, and less surface foam that tend to settle out to the bottom of the fermenter. The final flavor of the beer is depended on a great deal of the strain of lager yeast and temperatures. Some of the lager styles made from bottom-fermenting yeasts are Pilsners, Dortmunders, Märzen, Bocks, and American malt liquors.

2.2.5 Adjuncts

Adjuncts are materials other than malted barley that provide sources of carbohydrate and protein into wort, for example unmalted cereals such as corn, rice, rye, oats, barley, and wheat. Adjuncts are supplemented to the main mash ingredients often with the purpose for reduce costs, but sometime is generating additional characteristics. It illustrated result in beers with enhanced physical stability, superior chill-proof qualities, and greater brilliancy (Goldammer, 2000). Rice is a by-product of the edible rice milling industry wildly used in the production of beer, which insignificantly influence on the flavor. As brewing adjunct, the rice has a very neutral flavor, and aroma, which provided a light clean-tasting beer. (Goode and Arendt, 2006).

2.3 Types of beers

There are two main categories of beer in relation to fermenting yeast activities including top-fermentation, and bottom-fermentation beers.

2.3.1 Top-fermentation beer

Top fermentation beer is a type of beer using the top-fermenting yeast. Its hydrophobic surface causes the flocs to adhere to CO_2 and rise to the top of the fermenter during the fermentation process. This yeast ferments the beer quickly, provides a sweet, full bodied, and fruity taste. The types of top-fermenting beer are shown;

2.3.1.1 Wheat beer or Weizenbiere is the top-fermentation beer came from Bavaria made with at least 50% wheat malt. The typical wheat beer aroma is mainly influence by 4-vinyl-guajacol that contributes fruity, refreshing taste with a malty aroma, weak bitterness, and aroma of yeast.

2.3.1.2 Altbier is a dark hearty and little rustic beer. The altbier has an original gravity of 11.5 – 12% and an alcohol content of 4.8 – 5%v/v. The color ranges between 30 – 38 European Brewery Convention (EBC) units and the bitterness is 30 – 40 Bitterness Unit (BU).

2.3.1.3 Kölsch is a dry and pale beer, which can only producs in Cologne, Germany. The Kölsch beer has a light golden brown color of 7 - 10 EBC units, a bitterness of 16 – 35 BU and alcohol content of 4.6 – 5.1 %v/v. The Kölsch has a fresh and refreshing with a malty aroma of top fermentation.

2.3.1.4 Stout is a dark top-fermentation beer made from a mixture of pale malt and color malt such as black malt and caramel malt. Stout has a very strong burnt taste, which have bitterness about 40 – 50 BU, color is up to 200 EBC and alcohol content of 4.5 – 5.0%v/v.

2.3.1.5 Porter is a very strong dark beer about 300 EBC units and bitterness is 40 BU with an original gravity of 13 – 14% and 4.5 – 5%v/v of alcohol content.

2.3.2 Bottom-fermentation beer

The bottom-fermentation beer is a type of beer using the bottom-fermenting brewers' yeast. This yeast can flocculate and tend to settle out to the bottom of fermenter during the fermentation process. The final flavors of the beer depend a great deal on the strain of lager yeast and the temperatures of fermentation. The types of bottom-fermenting beer are shown;

2.3.2.1 Lager is a beer type which is definitely. Nowadays its accounts for 80 – 90% of the bottom-fermentation produced beers, which the common type of beer in the world. The word "lager" comes from German that mean "storage". The lager beer has a light golden color which has an original gravity is generally between 10.5 – 12.5%, alcohol content among 4.7 – 5.3% and moderately bitter around 18 – 23 BU.

2.3.2.2 Märzen is an Austrian beer type. The Märzen is classified as a "strong full beer" with an original wort extract of 13 – 14%, and alcohol content is 4.7 – 5.9%v/v. Two types of the Märzen consist of pale type with a color of 11 – 12 EBC units, and dark type with a color of 40 – 42 EBC unit.

2.3.2.3 Bockbier or Bock beer is very strong beer which has origins in Einbeck, Germany. The Bock beers brewed from 16 – 17% original wort extract. Thus, the longer of fermentation and lagering are very required. The Bock beers are pale (8 – 13 EBC unit), or dark (45 – 100 EBC unit) and have a high alcohol content of 7% v/v.

2.4 Beer production

There are 4 main steps for production of beer; malt production, wort production beer fermentation, and beer aging.

2.4.1 Malt production

Malting is the first step in beer production, which generates enzyme in germination grain kernel and causes certain changes in its chemical constituents. Barley malt is directed by a number of grain properties such as the content and composition of proteins, carbohydrates, endosperm structure, cell wall composition and the activities of hydrolytic enzymes during malting (Zhao *et al*., 2006). The processes of malt production consist of steps following;

2.4.1.1 Steeping

Steeping process has physiologically and biochemically changed of grains, which carried out by drainage of the water and resting in air to take place over a period of appropriate days. The correct combinations of water and air rest must be absorbed the water into the grain. At approximately 35% moisture content, the embryo within each kernel of grain is starting to germinate. The modification of rice or barley kernel is satisfactory if they have the appropriate moisture content at the end of steeping (Gamlath *et al*., 2008).

2.4.1.2 Germination

Germination is a process used to produce malt for the brewing. The germination is start during the air rests towards the end of the steeping stage. A technical term of “modification” is used to describe either all the physical and chemical change that occur when grains is converted into malt caused by the

degradation of the cell walls of starchy endosperm (Briggs, 1998). Gibberellic acid (GA) is synthesized by the embryo and diffused to the aleurone layer cell (see rice structural part in figure 2.1). Then, the aleurone layer is induced and amylolytic enzymes are released into the endosperm. Subsequently, the α-amylase and β-amylase convert the starch molecules of the grain into sugars that the embryo can use as food. The diagram and mechanism of germination of grains are illustrated in figure 2.4.

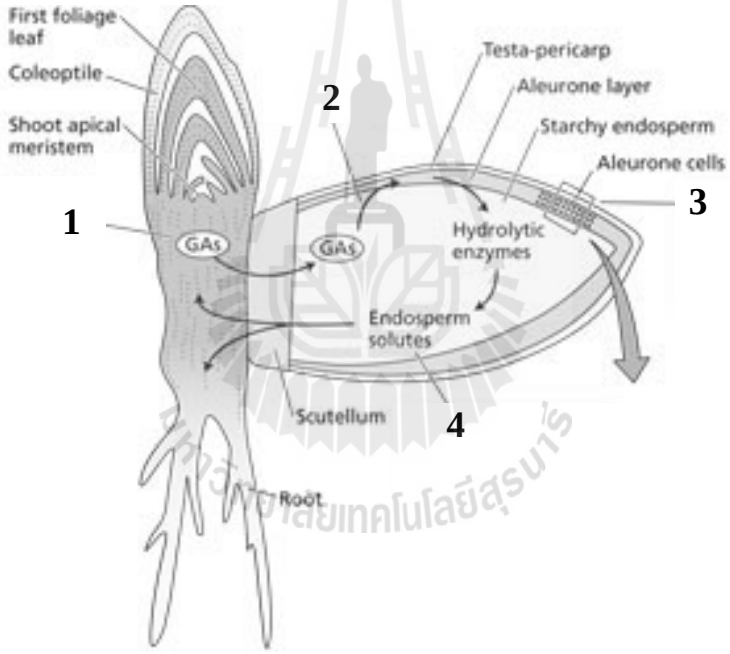


Figure 2.4 Mechanism of germination of grains.

1. GA is synthesized by the embryo and released into starchy endosperm via the scutellum.

2. GA diffuses to the aleurone layer.

3. Aleurone layer cells are induced to synthesize and secrete α-amylase and other hydrolases into endosperm.

4. Starch and other macromolecules are broken down to small molecule.

2.1.4.3 Kilning

Klining is the drying process designed for reducing the moisture content in germinated grain from over 43% to less than 5%. Water removal was done by passing hot air thought malt and germination of grain should be terminated. The reduction of moisture stabilizes the grain and allows to long-term storage, which inhibits all life process in malt for example, enzyme activity and modification in grain. During kilning, there is a development of color and increase the acceptable flavors. Color development results from the reaction between sugar and amino acids at high temperature to form melanoidins by Maillard reaction (Palmer, 2006). The mechanism of the Maillard reaction is illustrated in figure 2.5. After the kilning process, the rootlets are cut off and removed by put through a machine known as Deculmer to remove the culm or small rootlets that have emerged from each kernel during germination.

2.4.2 Wort production

The purpose of wort production is to provide the necessary conditions for fermentation of sugar from yeast to alcohol, and carbon dioxide. The initial components in malt must be converted to soluble fermentable sugar.

2.4.2.1 Malt milling

In order to provide malt enzymes, the malt must be broken into small fragments. The husks must be treated carefully and used as a filter material during lautering.

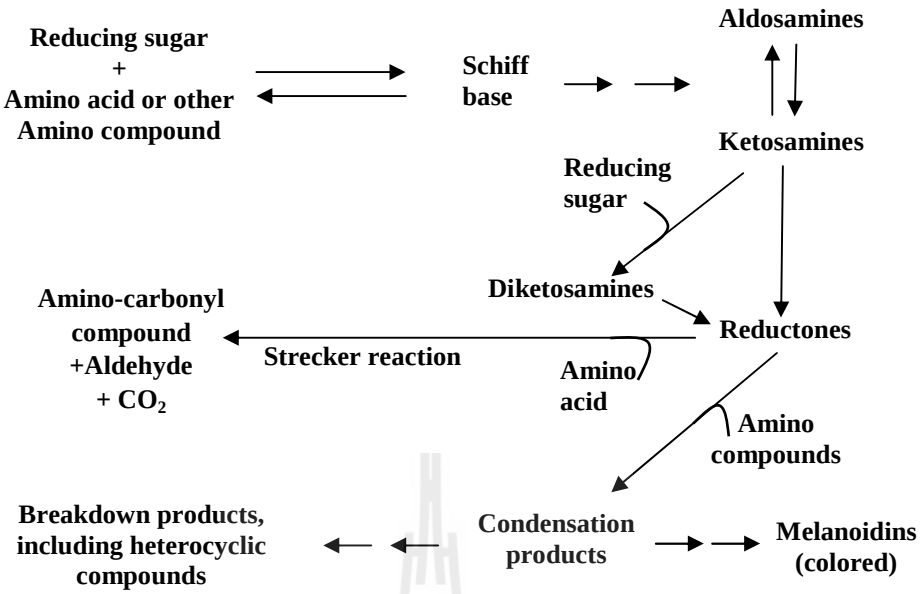


Figure 2.5 Formation of melanoidins, some flavor and aroma substance by Maillard reactions

Source: Briggs (1998)

2.4.2.2 Mashing

Mashing is the most important process in wort production. Grist malt is added to heat with purified water, carefully controlled time and temperature process. The malt enzymes break down the starch to sugar and non-fermentable sugar (dextrin) as well as the complex proteins of the malt is converted to simpler nitrogen compounds. During mashing, two amylase enzymes including α-amylase and β-amylase present the important function for starch hydrolysis. Alpha-amylase has optimal temperature in range of 70-75°C and β-amylase is around 55-60°C. The protease enzyme can work at temperature 45-50°C and FAN is determined as product of enzyme activity. Carbohydrate composition of various types of wort was showed in table 2.2.

Table 2.2 Carbohydrate composition of wort (g/100 mL).

Origin	Danish	Canadian	Canadian	German	UK	UK
Wort type	Lager	Lager	Lager	Lager	Pale ale	Pale ale
Concentration (°P)	10.7	11.9	11.55	12.0	10.0	10.0
Fructose	0.21	0.13	0.10	0.39	0.33	0.97
Glucose	0.91	0.87	0.50	1.47	1.00	0.60
Sucrose	0.23	0.35	0.10	0.46	0.53	-
Maltose	5.24	5.57	5.50	5.78	3.89	3.91
Maltotriose	1.28	1.66	1.30	1.46	1.14	1.39
Maltotetrose	0.26	0.54	1.27	-	0.20	0.53
Total dextrins	2.39	3.06	4.21	-	2.52	2.48
Total fermentable sugars	7.87	8.58	7.50	9.56	6.89	6.78
Total sugars	10.26	11.64	11.71	-	9.41	9.26
Fermentability (%)	76.7	73.7	64.1	-	73.3	73.2

Source: MacWilliam (1968)

The mashing methods can be divided into two main categories: infusion and decoction mashing. The infusion mashing is done at a single temperature without stirring, which requires high-quality and well germinated malt (Eaton, 2006). The decoction method is used as a series of different temperature with under-modified or enzymatically weak malt (Montanari *et al.*, 2005). The common rests occur at the temperature optima of enzyme:

45-50°C protein and β-glucanase rest,

62-65°C maltose production rest,

70-75°C saccharification rest,

75-78°C final mash temperature,

2.4.2.3 Lautering

At the end of the mashing process, the mash consists of wort mixture of undissolved substance called “spent grains”. The spent grains consist essentially of the husk, seedling and others materials, which are the source of protein and fiber that provide the benefits with human (Mussatto *et al*., 2006). Only wort is used for beer production, and spent grains must be separated. The mash is transferred to a straining or lautering vessel usually use cylindrical with a slotted false bottom. The liquid extract drains through the false bottom, and run off to the brew kettle. These extract through the grains is washed out as much of the extract as possible. The spent grains are removed and sold as animal feed.

2.4.2.4 Wort boiling and hopping are recognized as serving numerous of technologically important functions including:

a) Inactivation of residual enzymes activity after mashing process.

b) Sterilization of the wort to eliminate all bacteria, yeast and molds that could participate with the brewing yeast and possibly cause off-flavor.

c) Coagulation of excess proteins and tannins to form solid particles (trub), which are important for beer stability and foam. The key reaction during protein coagulation related with the destruction of disulfite bridges, which

convert to free thiol-groups. These can be reacted with thiol-groups of another proteins and peptides.

d) Formation of dimethyl sulfite (DMS) from the precursor S-methylmethionine (SMM) during wort boiling. However, the developments of DMS also appear through kilning and fermentation process (Figure 2.6). The DMS has a significant impact on beer flavor and is the most significant flavor compound from malt. Typically, the aroma of DMS is described as a cooked sweetcorn, tinned tomato or baked bean flavor (Bamforth, 2009). The accepte concentrations of DMS between the threshold level of approximately 30-100 μg/L (Hansen, 1999).

e) Extraction of the bittering compounds from hops.

f) Color formation by Maillard reaction.

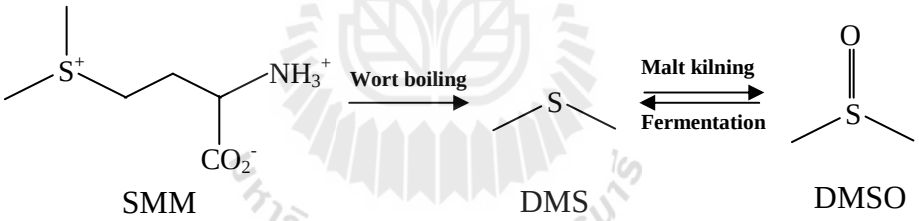


Figure 2.6 DMS formation during wort boiling, kilning and fermentation process

Source: Hughes (2009)

2.4.3 Fermentation and biochemistry of fermentation

To transform wort into beer, the sugar in wort must be fermented by yeast to ethanol and carbon dioxide. The consequence in the formation of fermentation and by-product has considerable effect on taste, aroma, and other characteristic properties of beer. For the fermentation of sugar and the carbohydrate metabolism such as glucose, fructose, maltose, and maltotriose are transported into the cell (figure 2.7). The enzyme invertase is responsible for the hydrolysis of sucrose. Glucose and fructose are converted in the cytoplasm and a series of complicate intermediate stage. Pyruvate is produced and ultimately converted to ethanol.

The conversion of glucose to 2 pyruvates within 10 intermediate stages is known as Glycolysis. Under the absence of oxygen, yeast converts pyruvate to ethanol via alcoholic fermentation shown in figure 2.8. However, the presence of oxygen fermentation is greatly inhibited or totally stopped called “Pasteur effect” the pyruvate is transported to mitochindria and breakdown via many intermediate stages to achieve high energy (36 ATP/mol), CO_2, and H_2O. Unfortunately, in the classical beer production process, wort aeration step is still necessary to ensure sufficient yeast growth, and a good fermentation performance. The wort aeration has a negative impact on wort qualities leads to oxidation which, can damage the flavor, and ethanol content (Depraetere *et al*., 2008).

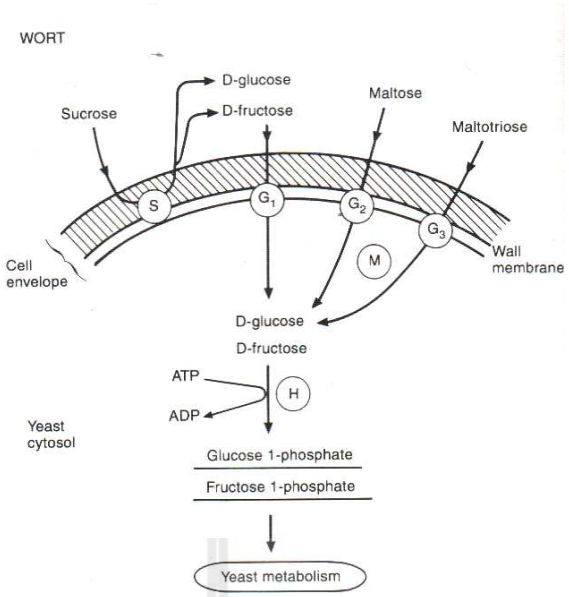


Figure 2.7 The uptake of wort fermentable sugars by yeast

Source: Lewis and Young (1995).

The yeast cells require many sources of nitrogen for the synthesis of cellular proteins and other cell compounds especially flavor-active compounds, and higher alcohol formation (O'Connor-Cox and Ingledew, 1989). Ingredew (1975) reported a rough distribution of nitrogenous compound in wort including 20% proteins, 30 – 40% polypeptide, 30 – 40% amino acid, and 10% nucleotide.

In wort, the main nitrogen sources for yeast metabolism are the individual amino acid, small peptide, and ammonium ions form from the proteolysis of barley malt proteins (Clapperton, 1971). The protein metabolism is therefore very important for the beer because many of metabolic products have a considerable effect on the flavor and stability.

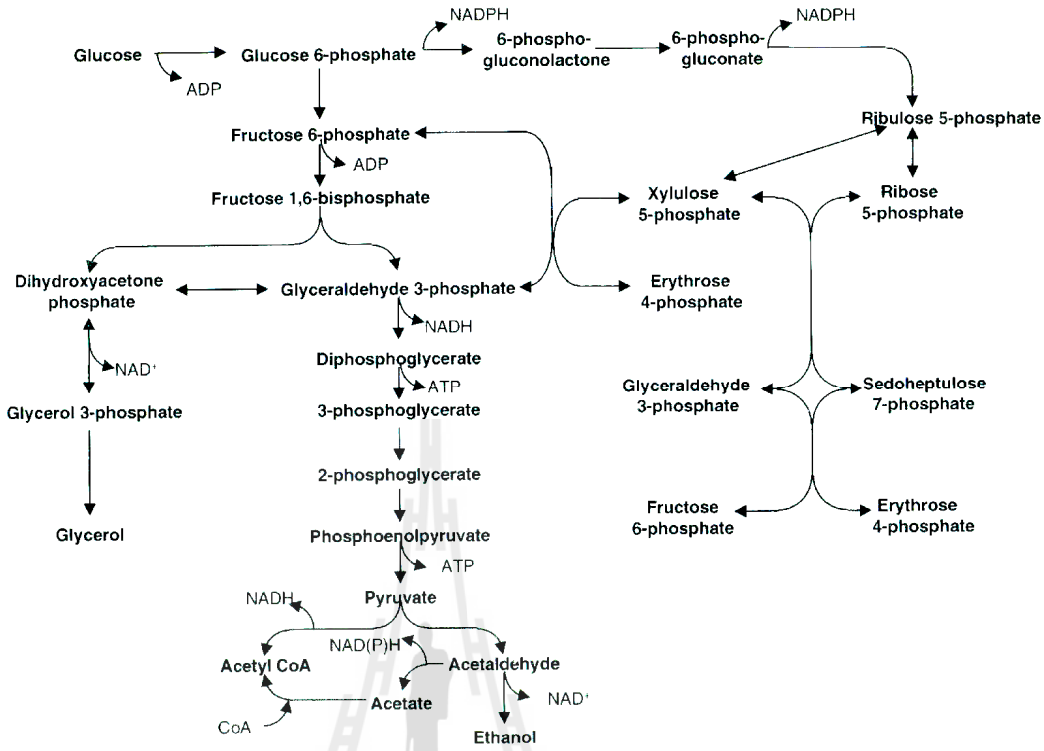


Figure 2.8 Pathways for dissimilation of glucose to ethanol in yeast via glycolysis and the hexose monophosphate shunt

Source: Boulton and Quain (2001)

Yeast has impact on the flavors, and aromas of beer. The flavors and the aromas of beer are very complex, being derived from a vast array of components that arise from a number of sources. These forms are the by-products during fermentation and maturation. The most notable of these by-products are ethanol and carbon dioxide, in addition, a large number of other characteristic flavors compounds are produced for example:

- acetaldehyde (green apple aroma)
- diacetyl (taste or aroma of buttery, or butterscotch)
- DMS (taste or aroma of sweet corn, or cooked veggies)
- fruity / estery (flavor and aroma of bananas, strawberries, apples, or other fruit)
- medicinal (chemical, or phenolic character)
- phenolic (flavor and aroma of medicine, plastic, Band-Aids, smoke)
- solvent (reminiscent of acetone, or lacquer thinner)
- sulfur (reminiscent of rotten eggs, or burnt matches)

2.4.4 Aging and finishing

Aging refers to flavor maturation at the end of fermentation, many undesirable flavor, and aroma of “green beer” or “immature beer” is presented. The purpose of aging is to reduce the levels of undesirable compounds including the diacetyl or the sulfur compounds as well as stabilization of beer. The diacetyl or vicinal diketones (VDKs) is the normal products of brewery fermentations that impart to beer characteristic aroma which provided a buttery flavor considered objectionable in lighter-bodied beer, if their concentration is 0.1 – 0.14 mg/L. During aging, yeast produced α-acetolactate then secreted into wort and converted to diacetyl and 2,3 butanedione.

After aging, clarification step, it is required to remove any remaining yeast as well as suspend particles of protein-polyphenol complexes and insoluble materials during cold storage. The common clarification techniques are used either or combination including sedimentation, fining, centrifugation, and filtration.

Carbonation is the process of adjusting the carbon dioxide (CO_2) replacement for the traditional rising to a specified concentration. Typical American lagers contain 2.5 – 2.8 volumes of CO_2 (1 volume is equal to 0.196% CO_2 by weight or 0.4 kg of CO_2/hL). The CO_2 concentration in beer can increase by increasing the headpressure of CO_2 also decreasing the temperature. The carbonation table was represented in table 2.3.

Table 2.3 Carbonation volumes related between headpressure (PSI) and temperature (°C)**.**

Temperature of Beer (°C)	Pound per square inch (PSI)																
	7	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**
3.0	2.15	2.24	2.34	2.42	2.52	2.62	2.72	2.80	2.90	3.00							
3.5	2.10	2.20	2.29	2.38	2.47	2.57	2.67	2.75	2.85	2.94							
4.0	2.05	2.15	2.25	2.34	2.43	2.52	2.61	2.70	2.80	2.89	2.98						
4.5	2.01	2.10	2.20	2.30	2.39	2.47	2.56	2.66	2.75	2.84	2.93	3.01					
5.0	1.97	2.06	2.16	2.25	2.35	2.43	2.52	2.60	2.70	2.79	2.87	2.96					
5.5	1.93	2.02	2.12	2.21	2.30	2.39	2.47	2.56	2.65	2.74	2.82	2.91	3.00				
6.0	1.9	1.99	2.08	2.17	2.25	2.34	2.43	2.52	2.60	2.69	2.78	2.86	2.95				
6.5	1.86	1.95	2.04	2.13	2.21	2.30	2.39	2.47	2.56	2.64	2.73	2.81	2.90	2.99			
7.0	1.82	1.91	2.00	2.08	2.17	2.26	2.34	2.42	2.51	2.60	2.68	2.77	2.85	2.94	3.02		
7.5		1.88	1.96	2.04	2.13	2.22	2.30	2.38	2.47	2.55	2.63	2.72	2.80	2.89	2.98		
8.0		1.84	1.92	2.00	2.09	2.18	2.25	2.34	2.42	2.50	2.59	2.67	2.75	2.84	2.93	3.02	
8.5		1.80	1.88	1.96	2.05	2.14	2.21	2.30	2.38	2.46	2.55	2.62	2.70	2.79	2.87	2.96	
9.0			1.85	1.93	2.01	2.10	2.18	2.25	2.34	2.42	2.50	2.58	2.66	2.75	2.83	2.91	2.99
9.5			1.82	1.90	1.98	2.06	2.14	2.21	2.30	2.38	2.45	2.54	2.62	2.70	2.78	2.86	2.94
10.0				1.87	1.95	2.02	2.10	2.18	2.25	2.34	2.41	2.49	2.57	2.65	2.73	2.81	2.89
10.5				1.84	1.91	1.99	2.06	2.14	2.22	2.30	2.37	2.45	2.54	2.61	2.69	2.76	2.84
11.0				1.80	1.88	1.96	2.03	2.10	2.18	2.26	2.33	2.41	2.48	2.57	2.64	2.72	2.80
11.5					1.85	1.93	2.00	2.07	2.15	2.22	2.29	2.37	2.44	2.52	2.60	2.67	2.75
12.0					1.82	1.89	1.97	2.04	2.11	2.19	2.25	2.33	2.40	2.47	2.55	2.63	2.70

Sources: modified from www.ebrew.com

2.5 Response surface methodology (RSM)

RSM is a collection of statistical and mathematical techniques useful for development, improvement, and optimization processes (Myers and Montgomery, 2002). The most general applications of RSM are in the particular situations where several input variables influence some characteristic of the process. Thus, characteristic is called the **"response"** or **"dependent variables"**. The input variables are sometimes called **"independent variables"**.

The RSM has been successfully used for various biotechnological and agricultural applications (Popa *et al*, 2007; Capanzana and Buckle, 1997; Vohra and Satyanarayana, 2002; Rodrigues *et al*., 2006; Wang *et al*., 2007). The field of RSM consists of the experimental strategy for exploring the space of the process or independent variables, empirical statistical modeling to develop an appropriate approximating relationship among the yield, the process variables, and optimization methods for finding the values of the process variables that produce desirable values of the response (Carley *et al*., 2004). Statistical modelling to develop an appropriate approximating model between the response *y* and independent variables $\xi_1, \xi_2, \ldots\ldots \xi_k$. In general, the relationship is:

$$y = f(\zeta_1, \zeta_2, \ldots\ldots \zeta_k) + \varepsilon \qquad \text{----------(1)}$$

The variables $\xi_1, \xi_2, \ldots\ldots \xi_k$ are usually called the **natural variables,** because they are expressed in the natural units of measurement such as degrees Celsius, pounds per square inch, etc. Generally RSM typically transforms the natural variables to **coded variables** $x_1, x_2 \ldots\ldots x_k$, which are usually defined to be dimensionless with mean zero, and the same standard deviation. In terms of the coded variables, the response function will be written as:

$$\eta = f(x_1, x_2, ...x_k) \qquad \text{----------} (2)$$

The first-order model is likely to be appropriate when the experimenter is interested in approximating the true response surface over a relatively small region of the independent variable space in a location where there is little curvature in *f*.

For the case of two independent variables, the first-order model in terms of the coded variables is

$$\eta = \beta_0 + \beta_1 x_1 + \beta_2 x_2 \qquad \text{----------} (3)$$

The form of the first-order model in equation is sometimes called a **main effects model**, because it includes only the main effects of the two variables x_1 and x_2. If there is an **interaction** between these variables, it can be added to the model easily as follows:

$$\eta = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{12} x_1 x_2 \qquad \text{----------} (4)$$

The second-order polynomial model is very useful in the RSM because it is very flexible and be able to take on a wide variety of functional forms. It often works well as an approximation to the true response surface. The data was fitted to a Taylor second-order polynomial equation:

$$Y = \beta_0 + \sum_{j=1}^{k} \beta_j x_j + \sum_{j=1}^{k} \beta_{jj} x_j^2 + \sum_{i<j}^{k} \beta_{ij} x_i x_j \qquad \text{----------} (5)$$

where:

Y = expected response

x = levels of the factor

β_0 = constant

β_j = linear terms coefficient

β_{jj} = quadratic terms coefficient

β_{ij} = interaction terms coefficient

The second-order model is widely used in RSM for several reasons:

1. The second-order model is very flexible which can take on a wide variety of functional forms. Also, usually work well as an approximation to the true response surface.

2. The second-order model is simple to estimate the parameters (β). The method of least squares can be used for this purpose.

3. There is considerable practical experience indicating that the second-order model works well in solving real response surface problems.

CHAPTER III

RESEARCH PROCEDURE

3.1 Materials

3.1.1 Rice and barley variety

Rice for malting and brewing in this research was hybrid medium short-grain rice CP13 provided by Charoen Pokphand Co. Ltd., Thailand. Barley malt for brewing in this research was *Baudin* species provided by Khon Kaen Brewery Co. Ltd., Thailand.

3.1.2 Microorganism

Yeast strain *S. cerevisiae* no.34 for bottom fermentation which is widely used in the brewing industrial from Technical University of Munich (TÜM), Weihenstephan, Germany.

3.1.3 Commercial enzymes

Commercial brewing enzymes were TermamylSC® as a heat stable α-amylase which has a standardized activity of 120 kiloNovo unit (KNU) and Neutrase® as a protease enzyme provided by Novozymes Inc.

3.1.4 Hops

Hops pallets was Hopsteiner P90 (4.3% α-acid) purchased from Hopsteiner, Mainburg, Germany.

3.2 Rice qualities

Proximate analysis of rice including moisture content, fiber content, total nitrogen content, fat content, ash content, and available carbohydrate were determined according to AOAC (1990). Percentage of rice germination was performed by EBC (1998).

3.3 Rice malt production

Malting schedule of rice was done by steeping and air rest as summarized in Table 3.1. Temperature of germination and relative humidity were controlled at 30°C and 99%, respectively in incubation chamber (Conviron E7/2). Germinated rice was collected on first, third, fifth, seventh, and ninth day of malting period and kilned at 50°C for 24 hours in each treatment. Rootlet and shoot of dried malt were eliminated and kept at 4°C in covered bucket. Qualities of rice malt including malting loss, free amino nitrogen (FAN), extract content, gelatinization temperature α-amylase and β-amylase activities were determined.

Table 3.1 Rice malting schedule

1st day- **2nd day**	**3rd day –** **4th day**	**5th day -** **6th day**	**7th day -** **8th day**	**9th day**
Steeping 8 h	Steeping 4 h	Steeping 10 h	Steeping 20 h	Steeping 24 h
Air rest 4 h	Air rest 8 h	Air rest 2 h	Air rest 4 h	
Steeping 8 h	Steeping 4 h	Steeping 10 h		
Air rest 4 h	Air rest 8 h	Air rest 2 h		

3.4 Optimization of mashing methods on wort qualities

3.4.1 Mashing time

The suitable mashing time in protease and saccharification rest (50 and 95 °C) was determined. The temperature-program was 45°C for 10 min, 50°C for 30 – 90 min, 63°C for 40 min, and 95°C for 30 – 90 min. The condition of mashing including: 3 days of germinated rice, 75% rice malt ratio, 0.25g/100g malt of commercial α-amylase and 0.25g/100g malt of protease enzymes. Spent grains were eliminated from wort. Qualities of wort including percentage of extract content, FAN, and filtrate volume were analyzed.

3.4.2 Optimization of mashing methods by RSM

The wort was done in mashing water bath (Julabo TW12) using 50 g of grist malt and 250 mL water by stirring at 100 rpm. The most appropriate temperature-time profile from previous experiment was carried out. The wort was eliminated from spent grain by percolated through filter paper No.1 (Whatman®, 110 mm diameter) and qualities of wort were determined. The experimental design was performed using Design Expert software version 7.1 (Stat-Ease Inc., U.S.A.). Optimization of beer production from rice malt was carried out by RSM based on face center central composite design which chosen to evaluate four factors: Germination time of rice (x_1), ratios of rice and barley malt (x_2), also commercial enzyme addition including Termamyl SC® (x_3), and Neutrase® (x_4), respectively (Table 3.2.).

Table 3.2 Process variables and their levels in the four-factor, three-level response surface design.

Variables	Codes	Variable levels		
		-1	**0**	**1**
Germination time of rice (days)	x_1	1	3	5
Rice malt (%)	x_2	50	75	100
α-amylase addition (g/100g malt)	x_3	0	0.25	0.5
Protease addition (g/100g malt)	x_4	0	0.25	0.5

A total of 52 experimental runs of 2 replications were completed as shown in Table 3.2. The dependent variables were determined: extract content (%) (y_1), FAN content (mg/L) (y_2), percentage yield of wort which adjusted to 12 degree plato (°P) (y_3), and fermentable sugar content (g/L) as a total of glucose, fructose, maltose, and maltotriose (y_4). These variables were expressed individually as a function of the independent variables.

The optimal condition of wort production was selected according to the following criteria: extract content of wort must be equal or more than 80%, FAN of wort must be a in range of 220-350 mg/L and yield of wort must be equal or more than 85%.

3.5 Laboratory scale fermentation

3.5.1 Inoculum preparation

The *S. cerevisiae* No. 34 for bottom fermentation was prepared by inoculating 2 full loops in 200 mL of YM medium (3 g/L yeast extract, 3 g/L malt extract, 5 g/L peptone and 10 g/L glucose). The medium was shaken at 28°C for 24 hours, 100 rpm. Yeast cell was separated by centrifugation at 4,000 rpm for 10 min (Sorvall RC 5C plus, U.S.A.) and transferred into 1 L of wort starter. Wort culture was aerated using aquarium pump for increasing cell number and temperature of inoculated wort was maintained at 14°C for 24 hours. Cell concentration was counted using Haematocytometer under microscope and calculated the volume of wort starter which needed to inoculated at 1.2×10^7 cells/mL of fermented wort.

3.5.2 Fermentation condition

The suitable mashing methods were selected to study the fermentation and beer production. The wort was prepared as described in 3.5.1 and adjusted to 12 °P. The wort was sterilized by boiling at 100°C for 60 min and further cooled to room temperature. Three liters of cast wort were transferred into 5 liters of sterilized fermenter (Sartorius Biostat, Germany). Bottom-fermenting yeast was pitched at 1.2×10^7 cells/mL at 14°C and stirred continuously at 50 rpm. Fermented wort was collected every 12 h for pH, viable cell count, FAN, ethanol, and reducing sugar analyses. Fermentable sugar content of fermented wort including glucose, fructose, maltose, and maltotriose concentrations were analyzed. Fermentation was stopped at 120 h, percentage of FAN and reducing sugar utilization were determined.

3.6 Fifty-liters brewing

The appropriate conditions from laboratory-scale mashing and fermentation were applied in 50 L brewing tanks by using 10 kg of grist malt and 50 L of water in mash tun. The temperature-time profile was used in accordance with laboratory-scale mashing. Sweet wort and spent grains were separated in lauter tun. Afterward, wort was boiled at 100°C for 60 min and hops pellet was added to obtain final beer bitterness for 25 BU (mg of bitter substance per liter of wort). Sterilized wort was transferred to the whirlpool for clarification and the wort was cooled to 14°C. Fermentation temperature and pitching rate were performed following the laboratory-scale brewing. Maturation step was done at 4°C for 1 week. Residual yeast was eliminated by centrifugation at 4,000 rpm for 10 min and 4°C (Sorvall RC 5C plus, U.S.A.) and carbonation of finished beer was done by direct addition of CO_2 into keg of beer and incubated at 4°C for 7 days. Physical properties of beer including beer color, sensory analysis, and volatile compound including alcohols, esters, acetaldehyde, DMS, and diacetyl of beer were analyzed.

3.7 Analysis

3.7.1 Analysis of malt qualities

3.7.1.1 Malting loss

Malting loss of malt was calculated according to Nirmala *et al.* (2000) by formula:

$$\%\text{Malting loss} = \frac{\text{wt. of 100 grains - wt. of 100 malt}}{\text{wt. of 100 grains}} \times 100 \qquad \text{----------} (6)$$

3.7.1.2 α- Amylase and β-amylase activity of malt

The α- and β-amylase activities were analyzed according to Iwouha and Aina (1997). Crude enzyme extraction was extracted by mixing 1 g of grinded malt with 9 mL of 50 mM Tris.HCl, pH 7.4 with 3mM $CaCl_2$, and 4 mM $NaCl_2$, then incubated at room temperature for 30 min, 15 min interval of shaking was done. The crude enzyme was separated by filtered through cotton and centrifuged at 4,000 rpm for 10 min at 4°C. The supernatant was maintained on ice until analysis.

The α-amylase and β-amylase activity assays were carried out by measuring of reducing sugar from starch hydrolysis as enzyme activities. For α-amylase enzyme assay, 0.5 mL of crude enzyme was incubated at 70°C for 5 min for activated α-amylase enzyme activity. Zero point 5 mL of substrate which contains 1% (w/v) potato starch in 50 mM acetate buffer (pH 5.5) with 0.003% $CaCl_2$ was added. The reaction was continued at 70°C for 10 min. For the β-amylase activity assay was the same procedure as the α-amylase enzyme assay, but crude enzyme was incubated at 50°C using substrate, which contains 1% (w/v) potato starch in 50 mM citrate buffer (pH 3.6) with 1 mM EDTA. One mL of 3,5-dinitrosalisylic acid reagent was supplemented. The mixture was boiled at 100°C for 5 min to stop both α-amylase and β-amylase enzyme reaction and then diluted with 10 mL water. The mixture was mixed thoroughly and measured the optical density of reducing sugar at 540 nm using spectrophotometer (Ultrospec 2000, Pharmacia Biotech).

Standard curve of glucose was accomplished by measuring the optical density of glucose solution (0, 0.2, 0.4, 0.6, 0.8, and 1 g/L) and procedure were done according to enzyme reaction assay.

3.7.1.3 Extract of malt: Congress mash

Extract of rice malt was performed according to EBC 4.5.1 (1998). Fifty grams of grinded rice malt were mixed with 200 mL of water and stirred at 100 rpm in mashing water bath (Julabo TW12) until mixture in the mash reaches 45°C. The mixture was maintained the temperature of 45°C for exactly 30 min. Afterward, the temperature of mash was raised 1°C per min for 25 min. When the temperature reached to 70°C, 100 mL of water at 70°C was added and incubated for 60 min. The mash mixture was cooled to room temperature and spent grain was percolated. Two-hundred mL of water was added to filtrate and soluble sugars of wort were determined on Hand Refractometer at 20°C. Extract content of wort was calculated according to the formula:

$$E_1[\%(m/m)] = \frac{P(M+800)}{100-P} \quad \text{----------(7)}$$

$$E_2[\%(m/m)] = \frac{E_1.100}{100-M} \quad \text{----------(8)}$$

where

E_1 = The extract content of sample (%m/m)

E_2 = The extract content of dry malt (%m/m)

P = The extract content in wort (°P)

M = Moisture content of malt (%m/m)

3.7.1.4 Free amino nitrogen (FAN) of malt

FAN of malt was determined using Ninhydrin method as described in EBC 7.6 (1998). Two mL of products from congress mash was mixed with 1 mL of color reagent (5 g of Ninhydrin in 1 L of water which contained 100 g Na_2HPO_4, 60 g KH_2PO_4, and 3 g of fructose) in a test tube. The test tube was placed in

boiled water for 16 min and then cooled to 20°C. Five mL of diluted solution (2 g KIO_3 in 600 mL water and 400 mL of 96% ethanol) was added and then measured the optical density at 570 nm. Blank was determined with 2 mL of deionized water. Glycine standard solution was checked using 2 mL of glycine solution (0.172 g glycine in 100 mL water and diluted to 1:100 before used). The FAN content in malt was calculated using the formula:

$$FAN_M = \frac{F.E'}{10.E_W} \qquad \text{----------(9)}$$

where:

FAN_M = Free amino nitrogen in malt (mg/100g DW)

F = Free amino nitrogen content in wort (mg/L)

E' = Extract of dry malt (%)

E_W = Grams of extract in 100 mL of wort (g/100 mL)

3.7.1.5 Gelatinization temperature of malt

The rice malt samples were grounded to 0.2 mm diameter. Gelatinization temperature was measured with a rapid visco analyser RVA Super 4 (Newport Scientific, Warriewood, Australia) as reported in Keßler *et al*., (2005).

3.7.2 Analysis of wort and beer qualities

3.7.2.1 Viable cell count

Viable cell count of yeast cell in fermented wort was done during fermentation step using Haematocytometer. The fermented wort was diluted with DI water to an appropriate cell concentration and then mixed with methylene

blue solution (0.1 g methylene blue in 100 mL water) before observation under microscope.

3.7.2.2 Reducing sugar of wort

The amount of reducing sugar from fermented wort was analyzed according to Miller (1951). One mL of diluted wort was mixed with 1 mL of DNS solution (10 g of 3, 5-dinitrosalicylic acid, 300 g of $KNaC_4H_4O_6$ in 200 mL of 2N NaOH and adjusted to 1 L with RO water). The mixtures were mix thoroughly and development of color was conducted by boiling the reaction tube for 5 min. The concentration of reducing sugar was calculated against standard of glucose concentration 0.2, 0.4, 0.6, 0.8, and 1.0 g/L.

3.7.2.3 Free amino nitrogen (FAN) of wort

Free amino nitrogen of wort and beer were determined by Ninhydrin method according to EBC 8.10 (1998). One mL of sample was diluted with DI water to 100 mL. Then, 2 mL of diluted sample was taken into test tube and 1 mL of color reagent was added. Test tube was placed in boiled water for 16 min and then cooled to 20°C. Five mL of diluted solution was added and measured the optical density at 570 nm. Blank was determined with 2 mL of deionized water. Glycine standard solution was checked using 2 mL of glycine solution. The FAN content was calculated the using formula:

$$FAN(mg/L) = \frac{A_1 \times 2d}{A_2} \qquad \text{----------} (10)$$

where:

A_1 = Optical density of test solution at 570 nm

A_2 = Mean observe density of standard solution at 570 nm

d = Dilution factor of sample

3.7.2.4 Extract content of wort

Extract content of wort was determined by measuring the soluble sugars of fermented wort during fermentation by Hand Refractometer at 20°C. Extract content of wort was calculated according to the formula:

$$E[\%(m/m)] = \frac{P(M+W)}{100-P} \qquad \text{----------(11)}$$

where

E = Extract content of sample (%m/m)

W = Amount of water per 100 g of malt (mL)

P = Extract content in wort (°P)

M = Moisture content of malt (%m/m)

3.7.2.5 Fermentable sugar and ethanol in wort and beer

Fermentable sugars in fermented wort for the period of fermentation step and beer including glucose, fructose, maltose, and maltotriose were established using High Performance Liquid Chromatography (HPLC) with Refractive Index (RI) detector (Agilent 1200, Agilent Technology Inc., U.S.A.). Standard mixture of glucose, fructose, maltose, and maltotriose concentration of 0.2, 0.4, 0.6, 0.8, and 1.0 g/L and standard ethanol concentration of 0.2, 0.4, 0.6, 0.8, and 1.0 %v/v were prepared. Samples were diluted with DI water at ratio 1:50 and filtered thought 0.45 μm filter paper (Whatman® 47mm diameter) before analysis. Ten μL of the sample was injected through a Vertisep™ OA (300x7.8 mm) column using DI water as a mobile phase at 0.8 mL/min flow rate. The temperature of column and detector were set at 30°C. Quantity of fermentable sugar and ethanols in samples were calculated using standard equation.

3.7.2.6 Volatile compounds and higher alcohols in beer

Volatile compounds and higher alcohols in finished beer were modified from EBC 9.12 (1998) using gas chromatography model CP 3800 coupled with a mass detector model 1200L Quadrupole MS/MS (Varian) by solid phase microextraction (SPME) technique. Samples were heated to 70°C and agitated continuously at 600 rpm for 20 min. The volatile compounds of samples were collected using Polydimethylsiloxane (PDMS) fiber syringe.

Volatiles compounds were collected by piercing septum vial by a septum piercing needle. After that, the fiber was exposed to the headspace of sample (20 mm above sample surface) for 20 min. This allowed the analytes from the solution to diffuse to the fiber. Then, the fiber was retreated inside the septum piercing needle and the fiber holder was removed from the sample. All analytes were absorbed on the fiber was desorbed by piercing GC inlet septum for 5 min.

The samples and standard were injected and separated on a DB-WAX column, 60 m x 0.25 mm i.d.: 0.25 μm film thickness (Agilent, U.S.A.). Column temperature-program was 35ºC 5 min and increased to 230ºC by 6 ºC per min (running time 37.5 min.). Mass detector conditions were electronic impact, EI0 mode at -70 eV. Source temperature was 220ºC, scanning rate 1 scan.s^{-1}. Mass acquisition range was 45-170. Carrier gas was helium at 1.0 mL/min. Identification of the volatile components of beer was carried out based on comparison of their GC retention times and mass spectra with authentic standards from NIST Mass pectral search Program for the NIST/EPA/NIH Mass Spectral Library version 2.0 (National Institute of Standard and Technology, U.S.A.).

Standard mixes of six levels including dimethyl sulfide (DMS), acetaldehyde, propanol, isoamyl alcohol, isobutanol, ethyl acetate, isoamyl acetate, 2-phenyl ethyl acetate, ethyl octanoate, ethyl decanoate and octanoic acid were prepared corresponding to Table 3.3 using 40% ethanol as a diluted solution.

Table 3.3 Concentration of standard volatile compounds

Volatile compounds	**Concentration levels (ppm)**					
	1	**2**	**3**	**4**	**5**	**6**
DMS	5	10	20	30	40	50
Acetaldehyde	5	10	20	30	40	50
Isoamyl alcohol	10	20	40	60	80	100
Propanol	10	20	40	60	80	100
Isobutanol	10	20	40	60	80	100
Ethyl acetate	10	20	40	60	80	100
Isoamyl acetate	5	10	20	30	40	50
2-Phenyl ethyl acetate	10	20	40	60	80	100
Ethyl octanoate	10	20	40	60	80	100
Ethyl decanoate	10	20	40	60	80	100
Octanoic acid	10	20	40	60	80	100

3.7.2.7 Color of beer

Color of beer was done by Spectrophotometric method which determined the absorbance of diluted sample at 430 nm within the linearity of the spectrometer. The colors for sample were calculated according to EBC8.5 (1998) using formula:

$$\text{Color (EBC units)} = A.\ f.\ 25 \qquad \text{----------} (12)$$

where:

A = absorbance of sample at 430 nm in 10 mm cell

f = dilution factor

3.7.3 Sensory analysis of beer

Sensory tests on aged beers were carried out using Hedonic Test with 12 assessors who have experience and familiar of beer drinking. The discussions for meaning of each attribute in score sheet were presented before beer tasting. The hedonic scale (5 levels) was designed for appearance, aroma, flavor, mouth-feel, and overall-impression. The score 0 was extremely dislike, 1 was dislike, 2 was normal, 3 was like, 4 was very like, and 5 was extremely like. For overall-impression, the scores of 0, 1, 2, 3, 4, and 5 were undrinkable, drinkable but not prefer to the next glass, drinkable and prefer to the next glass, good, very good, and excellent, respectively.

3.8 Statistical analysis

The statistical analysis was carried out by SPSS version 14 (SPSS Inc.). All chemical experiments were analyzed in duplicates. The statistical analysis was evaluated in Completely Randomized Design (CRD). Analysis of Variance (ANOVA) and means comparison by Duncan's Multiple Range Test (DMRT) were used to determine differences between mean at $p<0.05$.

CHAPTER IV

RESULTS AND DISCUSSIONS

4.1 Rice properties

Proximate analysis, percentage of germination and gelatinization temperature of rough rice (Hybrid rice CP13) used in this research were analyzed. The results were summarized in Table 4.1.

Table 4.1 Chemical composition of rice.

Nutrients	Chemical composition (%)
Starch	61.8
Moisture content	11.5
Crude fiber	8.8
Crude protein (N=5.95)	7.5
Ash	7.3
Crude fat	3.0
Germination (%)	96.0
Gelatinization temperature (°C)	95.0

Hybrid rice CP13 is medium long-grain rice that has a high productivity approximately 1 ton per hectare. Medium long-grain rice has benefits for malting and brewing as a result of the low viscosity problem, high level of water adsorption

(Bradee, 1977; Teng *et al.*, 1983) cause of the very high percentage of germination that about 96%. On the other hand, gelatinization temperature of rice was very high. Rice contained 3.0% of fat which higher than barley (2.0%). However, disadvantages for brewing were the formation of stale and off-flavor aldehydes from the oxidation of unsaturated free fatty acid (Schwarz *et al.*, 2002). Protein content of rice was 7.5% which less than barley, sorghum, and wheat, while starch was very high (61.8%). Rice contained 8.8% of crude fiber in form of rice husk that benefit for lautering process because of a filter aids properties in rice husk.

4.2 Effect of germination time on rice malt qualities

Germination of grain involves numerous metabolic pathways, particularly the starchy endosperm modification is a major mechanism of germination since starchy endosperm is accounted around 73 – 85% of grain portion (Bao and Bergman, 2001). Rice was germinated in the period of 1, 3, 5, 7, and 9 days. Qualities of rice malt including malting loss, extract content of malt, FAN of malt, α-amylase and β-amylase activities of malt were determined. The results were summarized in Table 4.2, compared with unmalted rice (control). Influence of difference malting period on the percentage of malting loss of malt was determined. Malting loss define as the loss of dry matter that occurred during malting result from the kilning and removal of the rootlets after germination. The results showed that malting loss was corresponding to the germination time. The rate of malting losses increased significantly during the germination time due to the lost of storage substance for rootlets and shoots production, elimination of longer rootlets, shoots of germinated rice, and the leaching of substances into the steep water. At the 7^{th} and 9^{th} day of germination provided the

29% and 38% of malting loss, respectively that mean the yield of rice malt were only 71% and 62%, respectively. In general, the inappropriate malting process occurred when the malting loss of malt is more than 20%, or the yield of malt is less than 80% cause to the higher production cost of malting and brewing. Hence, germinated rice in the 7^{th} and 9^{th} day was negligible.

The extract content illustrated the percentage of extracted compounds of grist malt from hot water used the soluble sugars was used as the major component. The extract content at the 1^{st} day of germination time was insignificantly difference with control ($P<0.05$). While, the extract content continuously increased during the 3^{rd} to 9^{th} day of germination. The maximum extract content occurred in the 9^{th} days of germination was 45.6%. However, the extract content of rice could not reach the typical brewing malt (80%) due to the low enzymes modification. At 70°C of congress mashing was used for the α-amylase activity that suitable for determined the extract content in barley malt. However, the gelatinized temperature of rice malt was undetectable. The congress mashing might be inappropriate for rice malt determination.

The FAN of malt considerably increased during the 3^{rd} to 9^{th} days of germination according to extract content due to the degradation of proteins to amino acids and small peptides by a range of proteolytic enzymes (Celus *et al.*, 2006; Enari and Sopanen, 1986). At the 9^{th} day of germination, it demonstrated the maximum FAN content that approximately 145 mg/100g of malt, which not notably variation with malt barley. Furthermore, the FAN of rice malt was higher than sorghum malt (70 mg/100g of malt) and consistent with wheat malt (143 mg/100g of malt) (Palmer *et al*, 1989).

Amylolytic enzymes in malt including α-amylase and β- amylase displayed the hydrolysis of starch into fermentable sugar that yeast can metabolize and converted to alcohol and other compounds (Evans, 2005). Unit of α-amylase and β-amylase activities of malt was expressed as the weight of reducing sugars generation (g) from starch hydrolysis in 10 min at the appropriate incubation temperature. The amylolytic enzymes of rice malt were not significantly different from control after the 1^{st} day of germination as a result of low extracts content. On the other hand, the α-amylase enzyme activity was highly increased after 3^{rd} day of germination, whereas β-amylase activity was increased and kept constant in 5^{th} day, after that it was significantly increased once more during the 7^{th} to 9^{th} day of germination. These results were related to extract content and α-amylase activity exhibited positively. The α-amylase and β-amylase activities of malt barley were 1.052 and 1.489 Unit/g malt, respectively, which are higher than that found in rice malt approximately 1.8 and 3.6 times, respectively. The β-amylase is exoenzyme, which catalyzed hydrolysis of α(1→4) linkages penultimate to non-reducing chain ends and released disaccharide maltose (the major enzyme in barley malt) while the α-amylase appeared in rice malt as the major amylolytic enzymes.

Table 4.2 Qualities of rice malt at difference malting period compared with control and malted barley.

Malt qualities	Control	Germinated time (days)					Barley malt
		1	3	5	7	9	
Malting loss (%)	0	3.99±0.73[a]	8.78±1.30[b]	17.57±1.68[c]	29.16±0.68[d]	38.92±0.98[e]	>20
Extract content (%)	11.26±0.01[a]	11.40±0.01[a]	15.40±1.31[b]	27.36±0.66[c]	41.70±0.01[d]	45.59±0.69[e]	≈80[f]
FAN (mg/100g malt)	5.22±0.43[a]	18.76±3.59[a]	44.70±4.88[b]	118.45±1.29[c]	136.41±4.88[d]	144.54±3.73[e]	≈150[e]
Gelatinization temperature (°C)	96.0	95.1	ud	ud	ud	ud	≈70
Enzyme activities (Unit/ g malt)							
α - amylase	0.015±0.001[a]	0.034±0.001[a]	0.312±0.007[b]	0.394±0.006[c]	0.484±0.023[d]	0.765±0.022[e]	1.052±0.020[f]
β - amylase	0.011±0.001[a]	0.032±0.001[a]	0.290±0.008[b]	0.294±0.025[b]	0.325±0.018[c]	0.411±0.016[d]	1.489±0.017[e]

Mean value of three replications of analysis ± standard deviation

The difference superscript letter between the columns were significant difference at $P \leq 0.05$

ud = undetectable

Enzyme activities (U) = weight of reducing sugars generation (g) from starch hydrolysis in 10 min per 1 g of malt

4.3 Influence of mashing time on wort qualities

Influence of mashing time for protease (50°C) and saccharification rest (95°C) on wort properties with sample prepared under condition of 3 day germinated malt and 0.25g/100g of commercial enzymes (zero-level of the variables) were carried out. The qualities of wort including FAN and extract content in finished wort were determined.

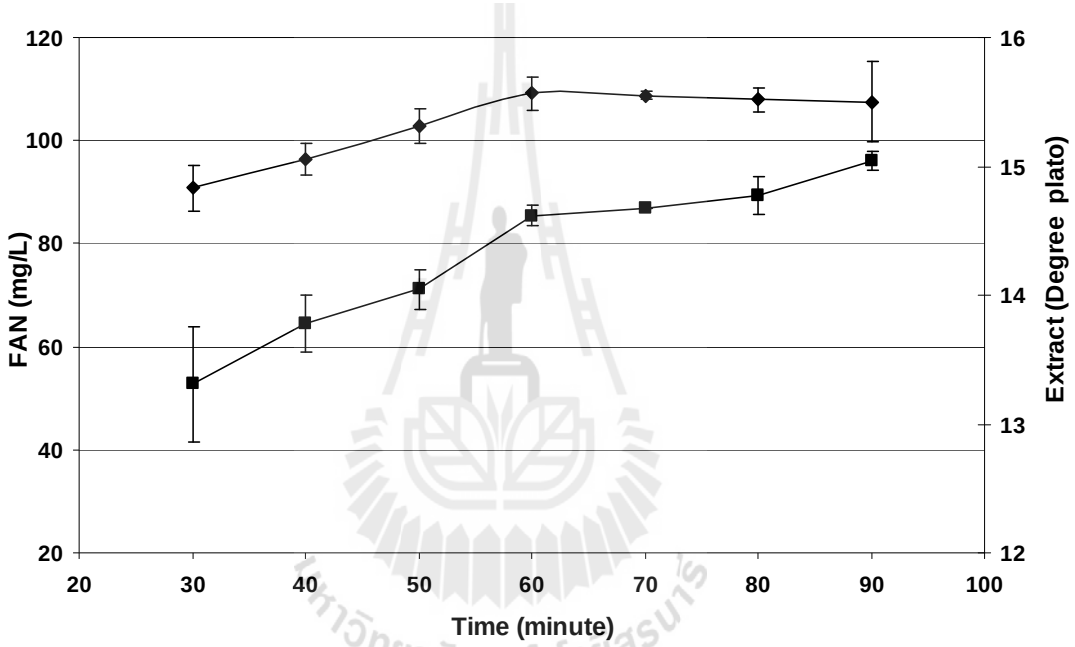


Figure 4.1 Time-course of FAN (♦) and extract content (■) in wort preparing by using 50% of 3 days germinated rice malt adding 0.25 g/100g commercial α-amylase and protease enzymes addition. Error bar indicated the standard deviation of 4 measurements.

Figure 4.1 showed the time-course of FAN and extract in wort from 3 days germinated rice (50%) using 0.25 g/100g malt of commercial α-amylase and protease enzymes. The commercial protease enzymes and α-amylase were supplemented at the

beginning of protease rest (50°C) and saccharification rest (95°C), respectively. The wort samples were collected every 10 min at 30 till 90 min of mashing time. As of the result show that, the rate of α-amino nitrogen liberation increased continuously from 30 minutes to 60 minutes at the maximum of 110 mg/L FAN from the activities of both commercial proteases such as proteolytic enzymes of rice and barley malt. Then, the FAN was stable after 60 minutes because of the limitation of protein in rice (7.5%). The result of extract content was consequent with the FAN, soluble sugars of wort increased to 14.6°P or approximately 86.5% extract, due to the activity of amylolytic enzymes. Hence, the temperature program for the experiment was 45°C 5 min, 50°C 60 min, 63°C 40 min, and 95°C 60 min. Gorinstein *et al.* (1980) studied the possibility of adjunct increasing by adding commercial enzymes to the mashing of 65:35 ratio of barley and sorghum malt. When added 0.1% commercial α-amylase (Termamyl 60L), the extract content was 72.0% and 72.5% extract content was found when added α-amylase mixed with β-glucanase enzymes (Cereflo 200L).

มหาวิทยาลัยเทคโนโลยีสุรนารี

4.4 Optimization of wort production using RSM

4.4.1 Model fitting and evaluation

The wort from rice malt at difference germination time, rice malt ratio, and commercial enzymes addition were investigated. The process variables cased the change in the following properties of wort including extract content, FAN, percentage of wort yield, and fermentable sugar content. The empirical model was constructed from experimental data and the significant of each variable term in the model was analyzed through *p* and *F* – value. The reliability of fitted models was determined by

analysis of regression coefficient including R^2, adjusted R^2, predicted R^2, and %C.V., respectively as represented in Table 4.3 and 4.4.

Table 4.3 illustrated *F*-value and *p*–value for each variable in the polynomial model. The *p*-value associated with a test statistic at least as extreme as the one that was actually observed that the null-hypothesis of observing a test statistic was true. The *p*–value lower than 0.05 indicated the dependent variables were significantly difference at 95% confident interval. In this experiment, the *p*-value of all models was lower than 0.0001, indicated that the model fitness was highly significant. The *p*-value for lack of fit showed the undesirable characteristic of the model, whereas *p* greater than 0.05 indicate that the lack of fit was not significant. It means that, the variation of the data around the fit model was small and the predicted models fitted the experimental data sufficiently.

Evaluations of independent parameter in fitted model for each dependent variable were determined. Individual rice malt ratio (x_2) and the commercial α-amylase addition ($x_{3)}$ showed the highly significant effect on the extract content of wort, while germination time (x_1) and the commercial protease addition (x_4) were not affect with the extract content at all (Table 4.3).

Three factors including germination time (x_1), rice malt ratio (x_2), and commercial protease (x_4) affected to the quantity of FAN. The germination time of rice and the rice malt ratio had interaction. Moreover, the quadratic terms appeared in germination time of rice (x_1^2) and commercial protease (x_4^2).

Table 4.3 *F*-value and *p*–value for each variable in the polynomial models.

Sources	Extract content		FAN		% Yield		Fermentable sugar	
	***F*-value**	***p*-value**	***F*-value**	***p*-value**	***F*-value**	***p*-value**	***F*-value**	***p*-value**
Model	11.08	< 0.001	43.67	< 0.001	11.11	< 0.001	70.92	< 0.001
Linear								
X_1	3.93	0.0605	159.65	< 0.001	3.49	0.0763	21.98	0.001
X_2	21.35	0.001	63.81	< 0.001	12.01	0.0024	257.62	< 0.001
X_3	11.23	0.0030	0.08	0.7794	25.94	< 0.001	54.97	< 0.001
X_4	-	-	124.96	< 0.001	-	-	-	-
Interaction								
$X_1 X_2$	-	-	23.27	0.0002	-	-	16.92	0.0005
$X_2 X_3$	7.78	0.0110	3.71	0.0719	5.98	0.0239	3.11	0.0932
Quadratic								
X_1^2	-	-	11.17	0.0041	-	-	-	-
X_2^2	-	-	-	-	8.14	0.0098	-	-
X_3^2	7.22	0.0151	3.38	0.0845	-	-	-	-
X_4^2	-	-	7.31	0.0156	-	-	-	-
Lack of fit	151.47	0.0640	3.48	0.4002	31.99	0.1385	1.92	0.5208

$p < 0.05$ = significant

Considering the yield of finished wort, rice malt ratio and commercial α-amylase addition were significant term while the germination time of rice malt was non significant term. This implied that the amount of α-amylase supplement in this experiment could overcome the less enzyme activity in germinated rice with no matter how long of germination time. The relations between rice malt ratio and commercial

α-amylase were described and proportion of rice malt addition appeared as the quadratic term.

The total fermentable sugar in wort was calculated as a summation of the fermentable sugar including glucose, fructose, maltose, and maltotriose. The result found that, three factors including germination time, rice malt ratio, and commercial α-amylase addition were strongly affected on fermentable sugar content while the interaction between germination time and proportion of rice malt were considered as insignificant term.

Table 4.4 showed the linear, interaction and quadratic regression coefficient of the predicted models. The mark of the regression coefficient specified trends effect to the dependent variables as positively and negatively. The germination time of rice malt demonstrated the positive impact to all the variables and showed the highest impact on the FAN. From the result, show that the germination time of rice can improve the overall qualities of wort. On the other hand, increasing the rice malt ratio caused the negative impact on the qualities of wort, especially on the FAN. Addition of the α-amylase has a positive effect to the yield, extract content, and fermentable sugar content but not effect on FAN at the same time as the commercial protease addition and the germinated rice grains exhibited the negative impact on FAN

The coefficient of variation (C.V.) is the standard deviation expressed as a percentage of standard error of predicted value to the mean value of observed response. A model can be considered logically reproducible if the C.V. is less than 10% (Ahmad *et al*., 2007). The fermentable sugar and the FAN content in wort were

in acceptable range of C.V., meanwhile germination time of rice and wort yield were greater than 10%.

. The mathematical model generated from the experimental data using Design-Expert software is expressed by the following quadratic equations:

$$\text{Extract content (\%)} = 123.56 + 2.86X_1 - 0.87X_2 - 63.66X_3 + 1.36X_2X_3$$

$$\text{FAN (mg/L)} = 297.1 - 62.19X_1 - 2.4X_2 + 268.1X_3 + 439.68X_4 + 0.45X_2X_3 + 9.37X_1^2 - 330.0X_3^2 - 485.23X_4^2$$

$$\text{Yield (\%)} = 6.16 + 2.15X_1 + 2.28X_2 - 24.73X_3 + 0.95\ X_2X_3 - 0.02X_2^2$$

$$\text{Fermentable sugar content (g/L)} = 138.07 - 5.27X_1 - 1.22X_2 + 9.07X_3 + 0.11X_1X_2 + 0.38X_2X_3$$

In order to confirm the suitable precision, the point prediction of the model was carried out by measuring the dependent variables at the point of the interests in triplicate experiments. The average values for each triplicate were compared to the predicted value or must be in range of 95% CI value which the upper and lower bound of the 95% confidence interval that surrounded the coefficient estimated for the center point. The point predictions of the models were completed (Table 4.5). All of 6 experimental values of the extract content and the percent yield of wort were in a range of 95% confident interval, conversely 2 experimental values of FAN were out of 95% confident interval range. As a result, the empirical models of extract content and the percentage yield were extremely confident and suitable for application while the empirical model of FAN was accepted.

Table 4.4 regression coefficient of the predicted models

Factors	Extract content (%)	FAN (mg/L)	Yield (%)	Fermentable sugar (g/L)
β_0	76.28	215.22	89.02	64.06
Linear				
β_1	5.71	55.69	4.30	5.89
β_2	-13.31	-35.20	-7.97	-20.15
β_3	9.66	-1.26	11.71	9.31
β_4	-	49.27	-	-
Interaction				
$\beta_1 \beta_2$	-	22.55	-	5.48
$\beta_2 \beta_3$	8.52	-9.01	5.97	2.35
Quadratic				
β_1^2	-	37.48	-	-
β_2^2	-	-	-11.83	-
β_3^2	-11.30	-20.63	-	-
β_4^2	-	-30.33	-	-
R^2	0.6784	0.9609	0.7353	0.9466
Adj. R^2	0.6172	0.9389	0.6691	0.9333
Pred. R^2	0.4570	0.9013	0.5285	0.8952
C.V.%	16.02	9.08	12.07	8.31

มหาวิทยาลัยเทคโนโลยีสุรนารี

4.4.2 Analysis of response surface

The 3-dimension response surface and the 2-dimension contour plots are the graphical representations of regression equation. They provided a method to reveal the relationship between responses and experimental levels of each variable and the type of interactions was between two test variables. The shape of the contour plots such as circular or elliptical, indicate whether the mutual interactions between the variables are significant or not. Circular contour plot indicates the interactions between the corresponding variables are negligible while, elliptical contour plot indicates that the interactions between the corresponding variables are significant (Zhong and Wang, 2010).

The relationship between independent and dependent variables was illustrated in 3-dimensional representation of the response surfaces and two-dimensional contour plots generated by the model of extract content, FAN, % yield, and fermentable sugar content. Two variables were depicted in one 3-dimensional surface plots, while the other variables kept at level zero.

มหาวิทยาลัยเทคโนโลยีสุรนารี

Table 4.5 The comparison of predicted model and experimental model

Runs[1]	Extract content (%w/w)		FAN (mg/L)		Yield (%)	
	Predicted Values	**Actual Values**	**Predicted values**	**Actual values**	**Predicted Values**	**Actual Values**
1	85.0 [78.9/98.16][2]	87.3	261 [235.5/286.2]	233	86.60 [75.7/97.5]	88.0
2	88.8 [79.4/98.2]	89.2	239 [210.1/267.5]	252	97.70 [89.62/105.73]	95.9
3	94.6 [83.5/105.8]	86.8	269 [243.0/294.0]	258	86.00 [76.7/95.3]	92.9
4	89.4 [80.7/98.1]	85.3	293 [268.5/317.0]	274	100.75 [91.8/109.7]	96.4
5	88.8 [77.6/99.9]	80.9	274 [244.2/302.8]	245	99.57 [90.2/108.9]	92.2
6	86.9 [73.7/100.0]	81.2	284 [258.5/309.1]	253	91.19 [80.3/102.1]	91.7

[1] Runs 1 ; X_1 = 1 day, X_2 = 50%, X_3 = 0.5g/100g malt, X_4 = 0.5g/100g malt

2 ; X_1 = 3 days, X_2 = 60%, X_3 = 0.5g/100g malt, X_4 = 0.5g/100g malt

3 ; X_1 = 5 days, X_2 = 50%, X_3 = 0.1g/100g malt, X_4 = 0.1g/100g malt

4 ; X_1 = 5 days, X_2 = 70%, X_3 = 0.4g/100g malt, X_4 = 0.2g/100g malt

5 ; X_1 = 5 days, X_2 = 90%, X_3 = 0.5g/100g malt, X_4 = 0.25g/100g malt

6 ; X_1 = 5 days, X_2 = 100%, X_3 = 0.5g/100g malt, X_4 = 0.5g/100g malt

[2] [95% CI low/ 95% CI high]

Figure 4.2A showed the effect of rice malt ratios and germination time of rice on extract content of wort. Germination time of rice highly impacted on extract content due to the chemical and physical modification of rice kernels by breaking down the starch granule to fermentable sugars (Kendall, 1995). On the other hand, the importance of germination time of rice slightly decreased when reduced the rice malt ratio (increase the barley malt proportion). However, germination time of rice did not impact to extract content when the barley malt was applied more than 50%. Therefore, the high level of amylolytic enzymes in barley malt could hydrolyze the macromolecules in both rice and barley malts whereas lesser enzymes in rice malt were negligible. Heat stable amylolytic enzymes increased the extract in wort. However, the extract content was constant when supplemented with α-amylase of more than 0.25g/100g malt, while the bacterial protease activity was not significant affect on extract content at any concentration of α-amylase.

Mashing program was done at 50°C for the degradation of low molecular weight protein particularly peptides and amino acids. The wort must be contained at least 200 mg/L of α-amino nitrogen for yeast propagation and fermentation. Protease activity from both of commercial enzyme and germinated malt improved the α-amino nitrogen liberation rate but not interacted between two variables. Mashing of 1^{st} day germinated rice with 0.50 g/100g malt protease could generate approximately 220 mg/L as the same amount found from 5^{th} day germinated rice without protease supplement. The polynomial curve of FAN down when the commercial protease was added, which indicated the rate of FAN generation slightly decreased as a result of limitation of protein body in rice malt. While, longer germinated rice malt had a higher impact on FAN than commercial enzyme addition. From the results, the FAN

in wort was noticeably increase when malt was used with a higher germination time. The result was in according with Markovic *et al.* (1995) who found the liberation of α-amino nitrogen in combination of barley protease extract and bacterial protease was more efficiency than single bacterial protease. While, the α-amylase had a little effect on the FAN by reason of the proteins enzyme increased the FAN in wort.

Response surface plot and contour plot of rice malt ratio and germination time at fixed commercial α-amylase and protease at 0.25 g/100g malt on percentage of wort yield when adjusted to 12°P was represented in figure 4.4A and 4.4B. Maximum filtrated wort obtained from 75% rice malt wort and germination time of rice enhanced the wort yield. In general, rice husk is a natural filter aids which has been shown an effective on brewing process including lautering and clarification step. Furthermore, rice husk can reduced the filtration time as well as increase the yield of filtrated wort (Villar *et al.*, 2004). However, the yield of wort decreased when the percentage of rice malt more than 80%. According to the extract content of wort was reduced due to increasing the level of rice malt.

Figure 4.6 represented the consequence of germination time of rice malt and rice malt ratios on the summation of fermentable sugar including glucose, fructose, maltose, and maltotriose. The quantity of malt barley ratio was impact to fermentable sugar content in wort due to the amylolytic enzymes activity as same as extract content. Longer germination time of rice malt also affected to fermentable sugar, by reason of internal structure of rice malt endosperm, amylolytic and proteolytic enzymes are produced during germination.

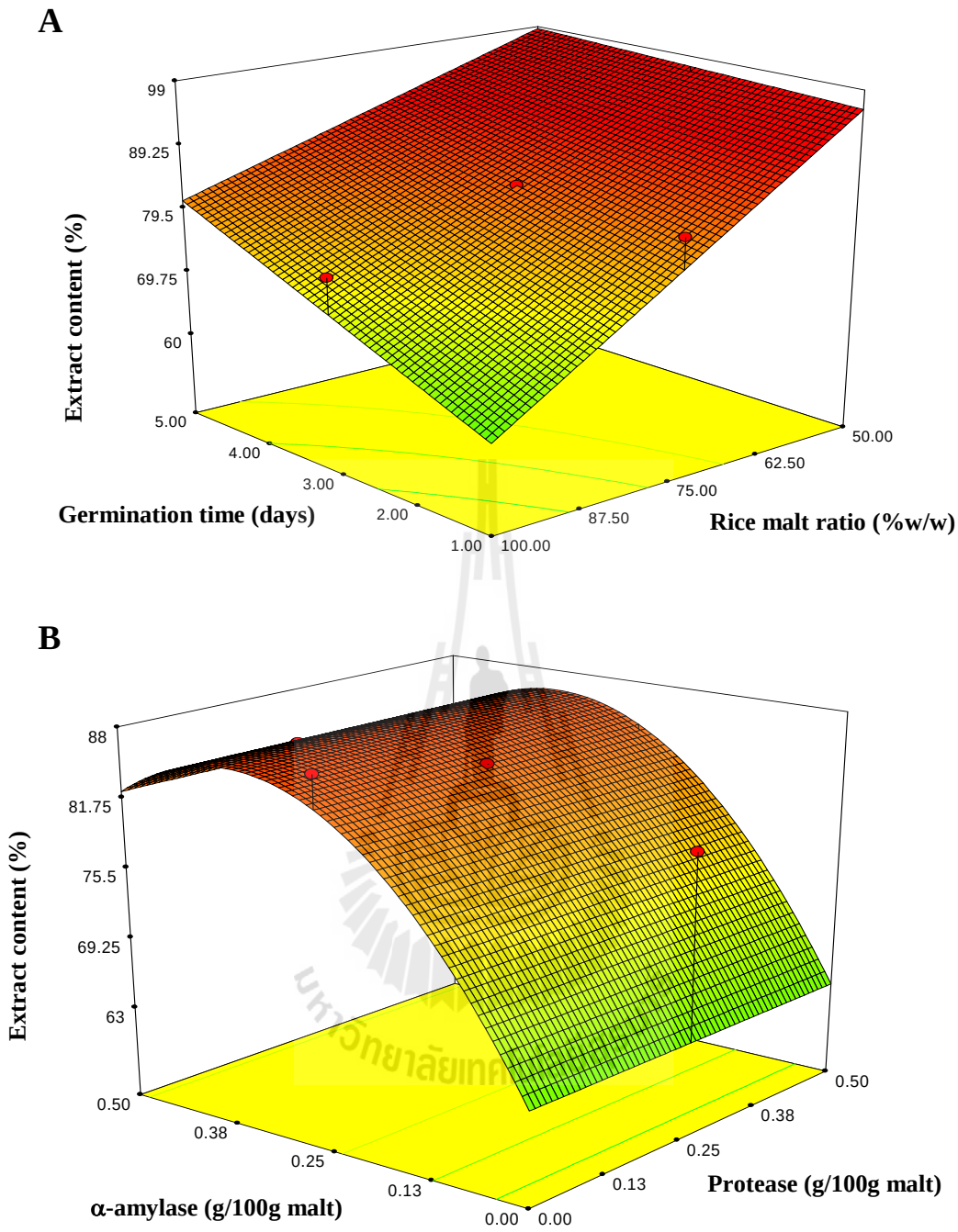


Figure 4.2 Response surface plots of extract content on (A) germination time of rice and rice malt ratio at fixed commercial α-amylase and protease of 0.25g/100g malt and (B) commercial α-amylase and protease at fixed germination time of rice at 3 days and rice malt ratio at 75%.

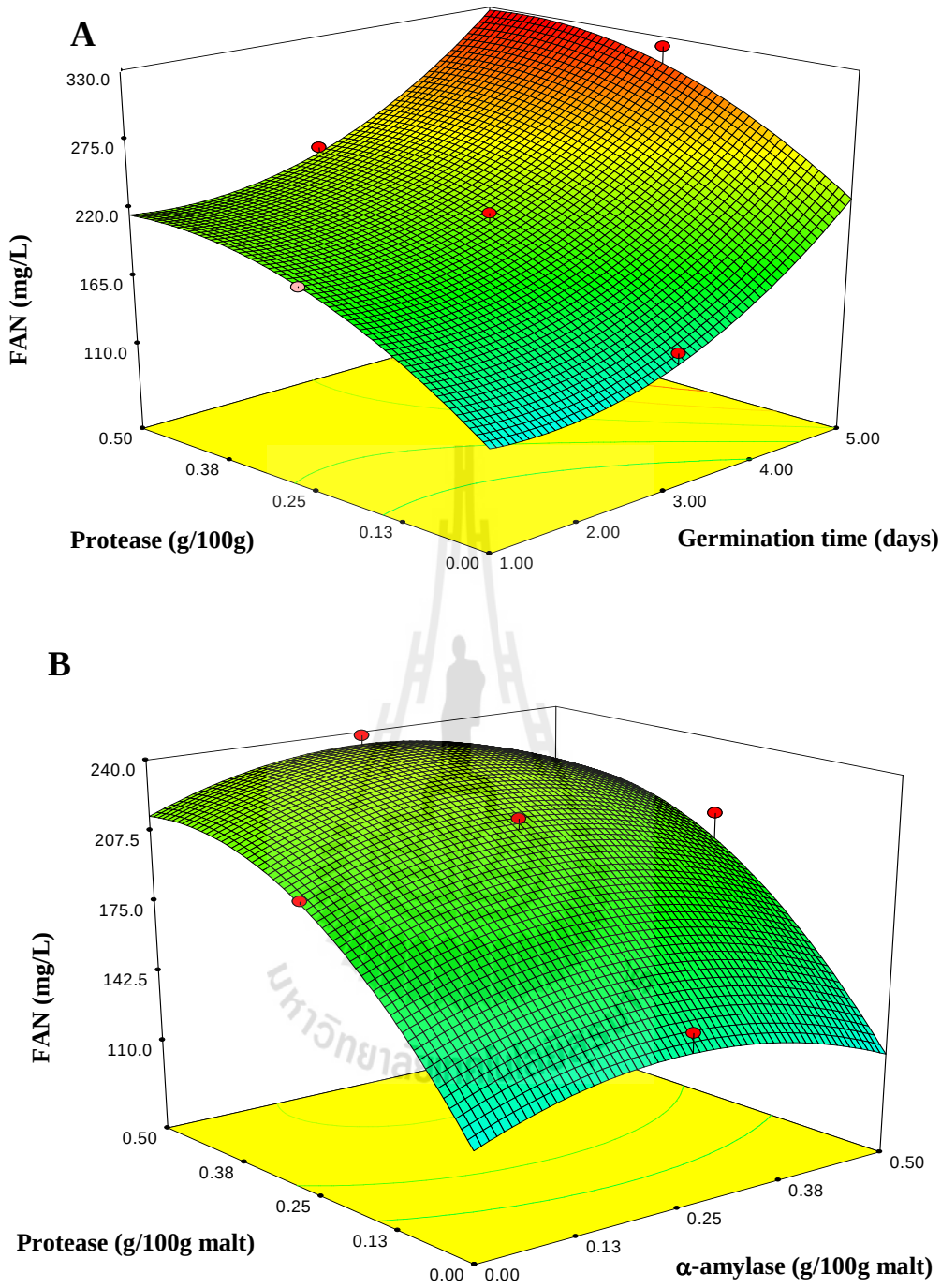


Figure 4.3 Response surface plots of FAN on (A) germination time of rice and protease at fixed commercial α-amylase of 0.25g/100g malt and germination time at 3 days and (B) commercial α-amylase and protease at fixed germination time of rice at 3 days and rice malt ratio at 75%.

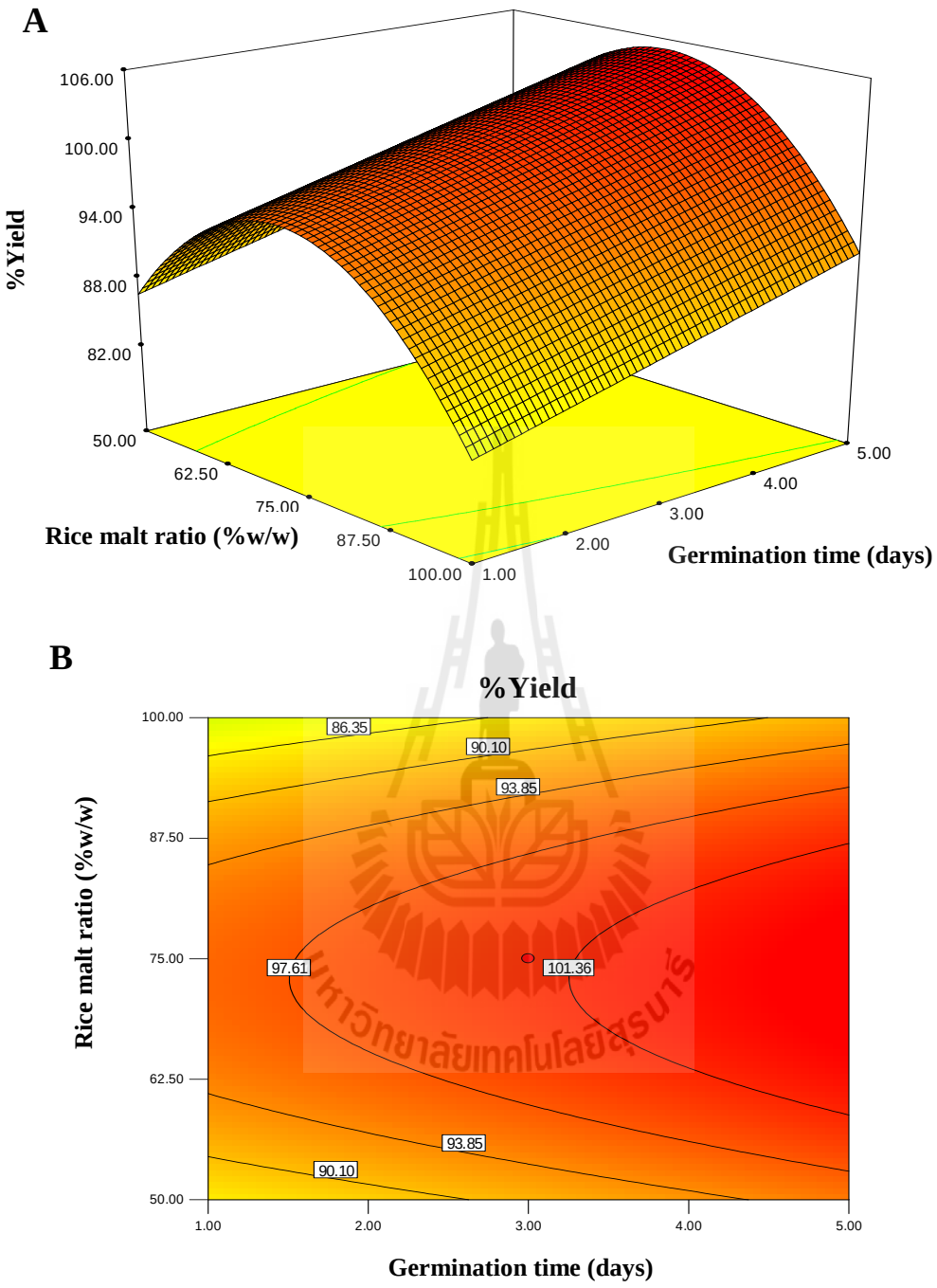


Figure 4.4 Response surface plot (A) and contour plot (B) of %yield with various rice malt ratio and germination time on fixed commercial α-amylase and protease at 0.25g/100g malt.

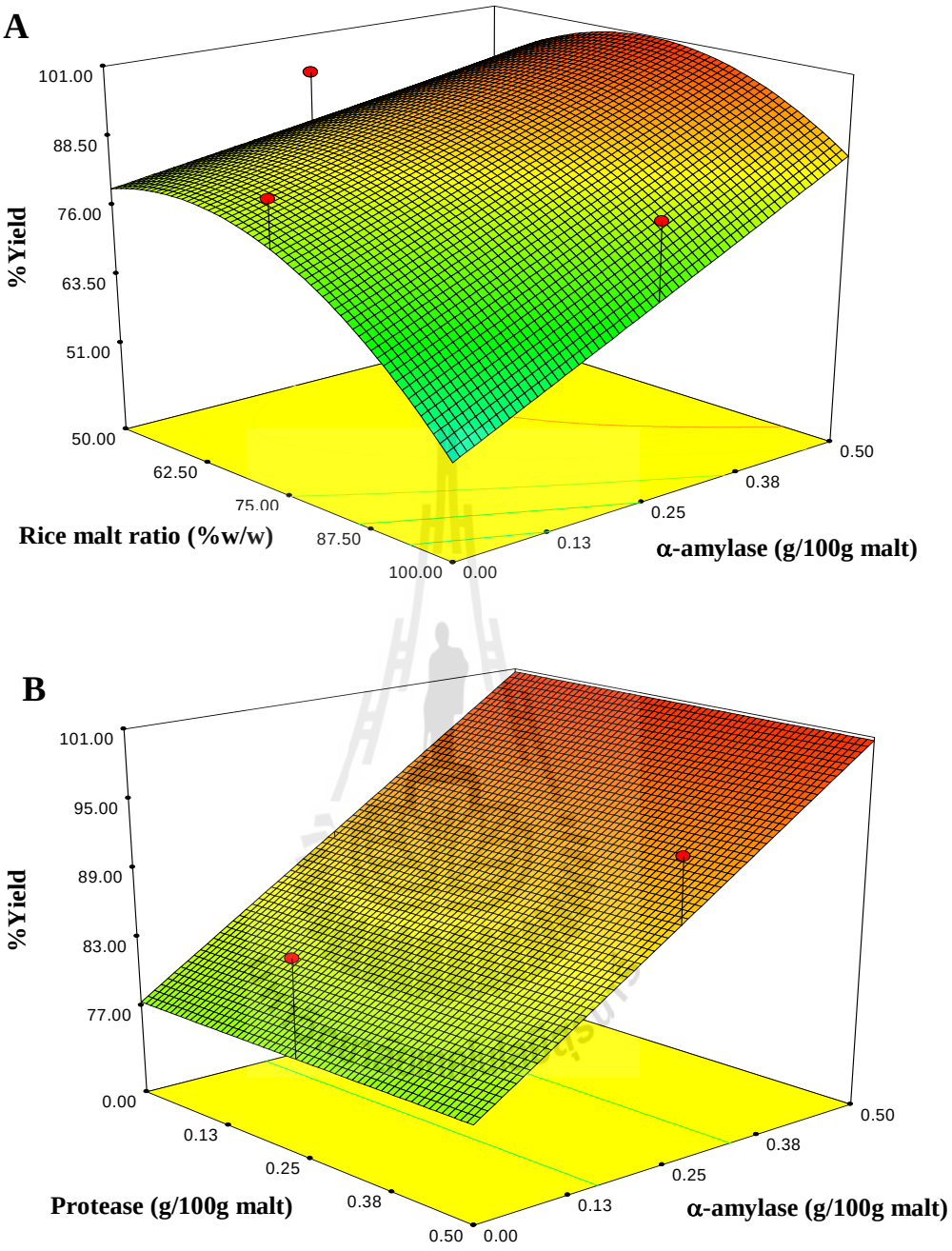


Figure 4.5 Response surface plots of %yield on (A) rice malt ratio and α-amylase at fixed germination time of rice at 3 days and protease at 0.25g/100g malt on yield and (B) commercial α-amylase and protease at fixed germination time of rice at 3 days and rice malt ratio at 75%.

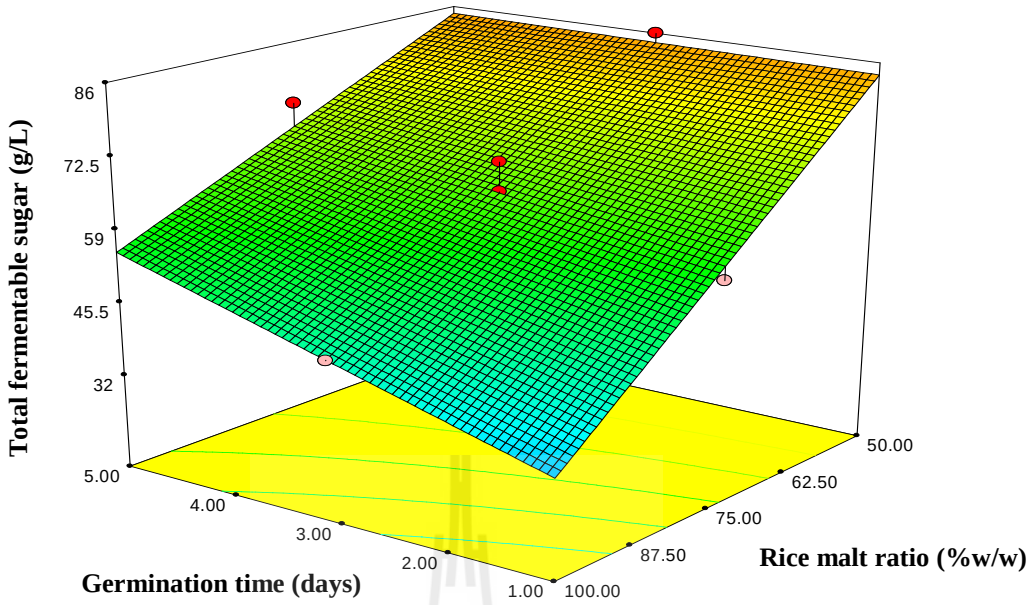


Figure 4.6 Response surface plot of total fermentable sugar content on germination time and rice malt ratio of rice at fixed commercial α-amylase and protease at 0.25g/100g malt.

4.4.3 Optimization of wort production

The numerical of the appropriate conditions for mashing process based on germination time and rice malt ratios were demonstrated in Table 4.6. The predicted criteria of wort were selected. Extract content must be equal or more than 80%, the FAN of wort must be in range of 220-350 mg/L and yield of wort should be equal or more than 85% whereas fermentable sugar was negligible. Desirability indicated the desirable range of each response (Myers and Montgomery, 2002) equal to 1.000, which specifies the treatment was appropriated. Rice malt from 5th days of germination showed the maximum rice malt ratio at 90% with 0.40 g/100g malt of both enzymes supplementation. Furthermore, only 1st and 3rd days of germination only 70% ratio of rice can achieved the accept wort qualities with 0.50 and 0.40 g/100g malt of

commercial enzymes addition, respectively. Internal structure properties as the gelatinization temperature and enzymes generation were modified during the germination of grains that is able to reduce the commercial enzymes addition.

Table 4.6 Numerical optimization of wort production base on germination time and ratio of rice malt as well as predicted value of wort qualities.

Batch	Germination time (days)	Rice malt (%)	α-amylase (g/100g)	Protease (g/100g)	Extract (%)	FAN (mg/L)	Yield (%)
1-50	1	50	0.50	0.50	85	261	86.6
1-70	1	70	0.50	0.50	81	207	96.0
3-50	3	50	0.30	0.25	89.8	251	86.3
3-70	3	70	0.40	0.40	83.7	224	96.5
5-50	5	50	0.10	0.10	94.6	269	86.0
5-70	5	70	0.25	0.25	84.7	311	90.2
5-90	5	90	0.40	0.40	82.9	308	93.4

มหาวิทยาลัยเทคโนโลยีสุรนารี

4.5 Laboratory scale fermentation

4.5.1 Fermentable sugar content in fermented wort

Total 7 treatments of mashing programs in various germination time and ratio of rice malt were done. The wort was sterilized at 100°C for 1 hour and hops were added to reach 25 BU. Fermentable sugars of wort in each treatment including glucose, fructose, maltose, and maltotriose were analyzed by HPLC and sugars ratios were determined (Table 4.7). Modification of rice grain was capable of improving the amount of total fermentable sugars in wort. Fifty percent of the 5^{th} days of germinated

rice (5-50 wort) illustrated the highest fermentable sugar content in wort at 81.52 g/L. On the other hand, fermentable sugar concentrations were 73.72 and 70.09 g/L on the 1st and 3rd day of germination, respectively. Furthermore, the increased of maltose and glucose at 33.97 and 26.79 g/L showed the enhancement of fermentable sugars content in wort. The results indicated that the modification of rice malt was higher impact on fermentable sugars concentration than the commercial enzymes addition at 50% rice malt ratio because of the amylolytic enzymes activity in malted barley. However, the commercial enzymes had more effect when the rice malt ratio reached up to 70% due to the insufficient of enzymes at high ratio of rice malt. Addition of commercial enzymes increased the concentration of fermentable carbohydrate, soluble protein, and free amino nitrogen whereas all components took advantage in decreasing of wort viscosity, higher filtration rate, increasing of yield, and adding more amount of adjuncts (Denault *et al.*, 1981; Goode and Halbert, 2003).

Total fermentable sugars of wort from the 5th day of germination at 50% were similar as Canadian lager wort, which contained 85.80 g/L fermentable sugars at the same wort concentration (12°P). Whereas, experimental wort was found lesser than German lager wort of 95.60 g/L. Maltose in German lager wort was very high at 57.80 g/L or 56.5%, which was more than experimental wort approximately 1.5 - 3 times. While glucose was lower at 14.7 g/L or 14.6%. The amount of glucose and maltose in Canadian lager were about 5.0 and 55.0 g/L, respectively (MacWilliam, 1968; Hoekstra, 1974). The activity of β-amylase in barley malt was the main reason of the high level of maltose. High concentration of glucose found in wort was from the α-amylase of both native and commercial addition. The difference quantity and ratio of

fermentable sugar had impact on yeast metabolism, fermentation parameters, and qualities of final beer after fermentation.

4.5.2 Effect of germination time, commercial enzymes and malt barley addition on fermentation profile

Time-course of viable cell count, reducing sugar, FAN utilization, and ethanol production as well as fermentable sugar consumed (glucose, fructose, maltose, and maltotriose) during fermentation were illustrated in figure 4.7. The suspended cell concentrations of all treatments were increased sharply within 36 hours and then decreased gradually until the finish of fermentation. Whereas, the FAN in wort was consumed rapidly from 0 to 60 hours. Then, the FAN was stable for every treatment. Lekkas *et al.* (2007) reported that amino acids group A, the fast absorbtion amino acid including glutamic acid, aspartic acid, asparagine, glutamine, serine, threonine, lysine, and arginine were disappeared within 15 hours in wort fermentation. Subsequently, intermediate absorption amino acid, or amino acid group B including valine, methionine, leucine, isoleucine, and histidine were utilized slowly and gradually (Perpête *et al.*, 2005). Pickerell (1986) reported that the initial wort FAN had an effect on the FAN uptake rate, the sugar utilization rate, and ethanol production rate. Furthermore, direct influence on linear growth rate and mass increase the number of the yeast cells.

Table 4.7 Fermentable sugars content and sugars ratio of experimental wort.

Treatments	Fermentable sugar content (g/L)				Total (g/L)
	Glucose	Fructose	Maltose	Maltotriose	
1-50	17.87[b] (25.50)	2.42[d] (3.45)	31.72[e] (45.26)	18.08[f] (25.79)	70.09[e]
1-70	16.74[a] (25.67)	1.11[a] (1.71)	27.45[d] (42.09)	19.91[g] (30.54)	65.21[d]
3-50	21.75[f] (29.51)	2.39[d] (3.24)	31.66[e] (42.94)	17.92[e] (24.31)	73.72[f]
3-70	19.34[c] (33.15)	1.73[b] (2.97)	22.28[b] (38.19)	14.99[c] (25.69)	58.33[b]
5-50	26.79[g] (32.87)	3.27[e] (4.01)	33.97[f] (41.68)	17.49[d] (21.45)	81.52[g]
5-70	20.55[d] (34.65)	1.73[b] (2.92)	22.89[c] (38.58)	14.15[b] (23.85)	59.32[c]
5-90	21.34[e] (38.67)	1.83[c] (3.32)	19.02[a] (34.46)	13.00[a] (23.55)	55.19[a]

() = sugar ratio (%)

Glucose and fructose as the monosaccharide were utilized and completed at 36 hours of fermentation period for every treatment. Maltose is the major sugar, which did not significantly decrease during the start of fermentation, presumably because of the maltase permease or the repressed of glucose and fructose utilization (Patel and Ingledew, 1973). The presence of glucose had effect on *Mig1* complex and

MAL-activator proteins like *MAL63* transcription seem to be the major step in specific glucose repression of maltose metabolism (Novak *et al.*, 2004). The rapid utilization of maltose was occurred between 36 – 80 hours of fermentation eventually resulting in 80 – 85% of maltose utilization, while the decrease of fermentable sugar was positive with the decrease of the reducing sugar. However, maltotriose, the trisaccharide fermentable sugar was not utilized leading to the low ethanol production. *AGT1* permease was the unique α-glucosidase transporters, which requires for the active transport of the maltotriose across the plasma membrane (Alves *et al.*, 2007). Zheng *et al.* (1994) studied the factors which influencing on the maltotriose utilization of brewing yeast. It was found that the maltotriose uptake was dependent on the yeast strains, the pitching level, fermentation temperature, pH value of wort, and ethanol level whereas quantity and type of ions were not affected. The maltotriose uptake could be enhanced at the 21°C of fermentation temperature with a higher pitching level and agitation. In addition, the maltotriose uptake ability of the lager strains was better than the ale strains. On the other hand, the uptake rates of glucose and maltose in wort showed no differences between ale and lager yeast. The inefficiency of the maltotriose utilization leads to one of the problem in the brewery, for example atypical beer flavor profiles in finish beer.

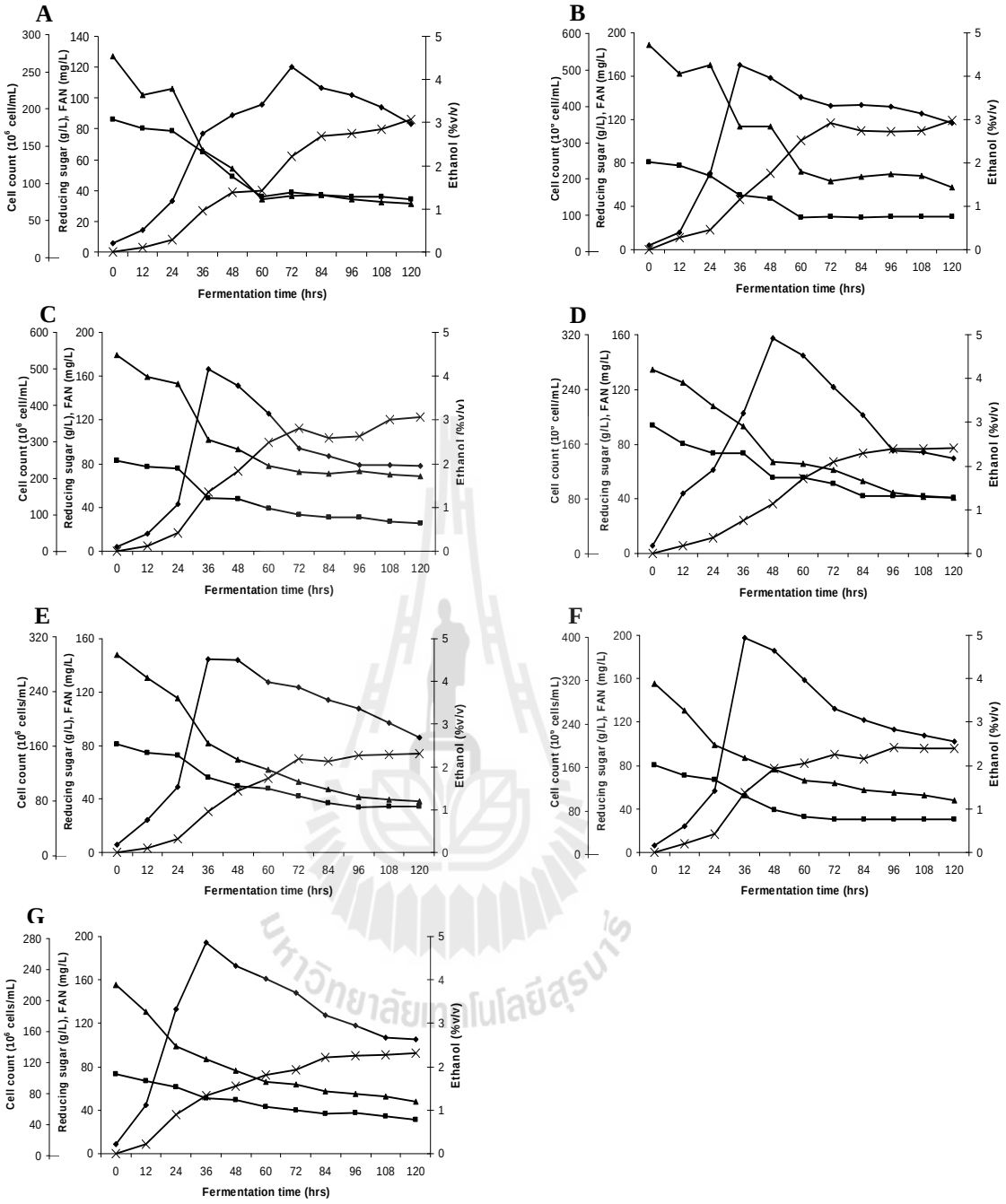


Figure 4.7 Time-course of viable cell count (●), reducing sugar (■), FAN (▲), and ethanol (x) during fermentation at different mashing conditions A) 1-50 wort, B) 3-50 wort, C) 5-50 wort, D) 1-70 wort, E) 3-70 wort, F) 5-70 wort and G) 5-90 wort

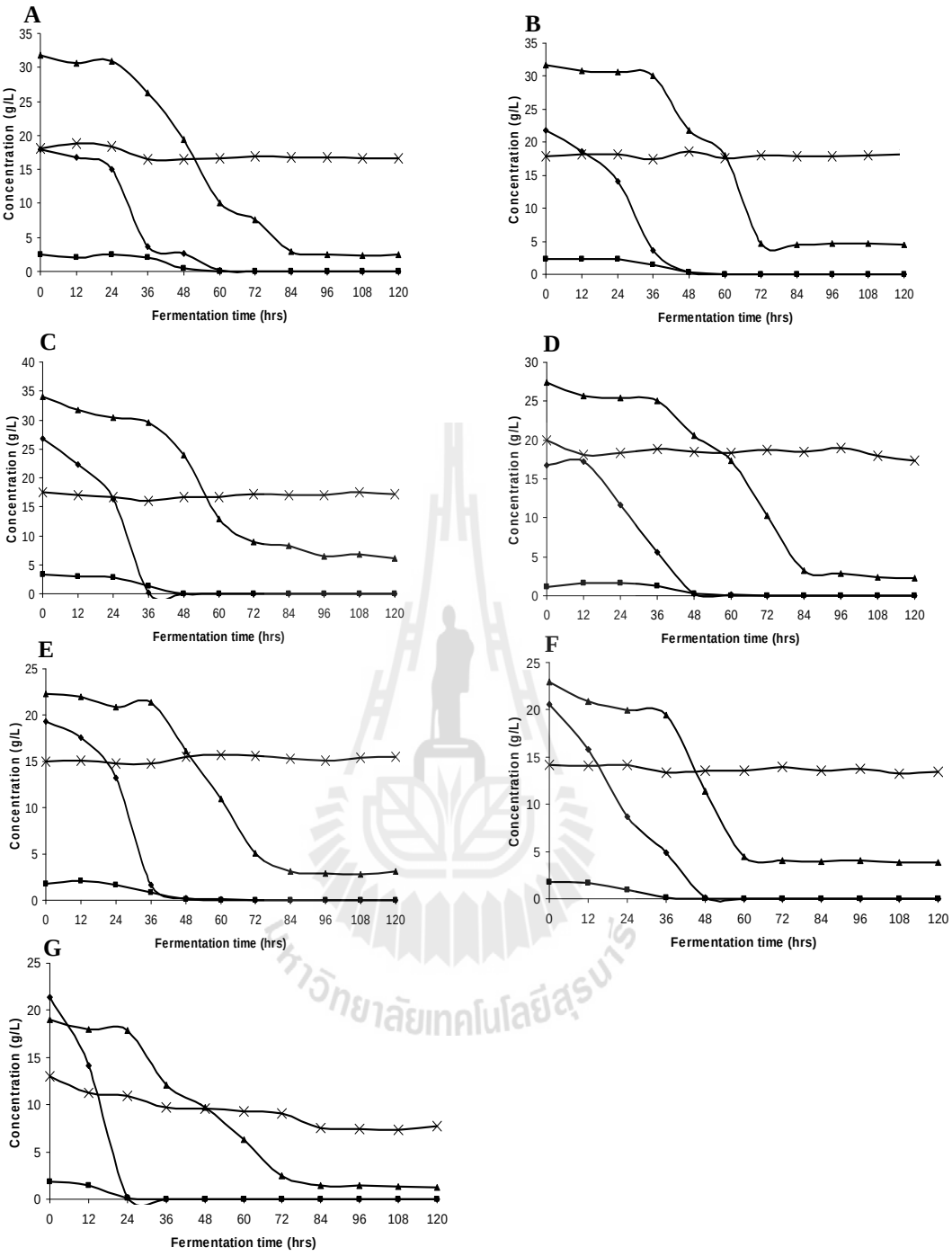


Figure 4.8 Time-course of fermentable sugar including glucose (♦), fructose (■), maltose (▲), and maltotriose (x) during fermentation at different mashing conditions A) 1-50 wort, B) 3-50 wort, C) 5-50 wort, D) 1-70 wort, E) 3-70 wort, F) 5-70 wort and G) 5-90 wort

Table 4.8 illustrated the percentage of the FAN and reducing sugar uptake after finishing of the fermentation at 120 hours. Percentage of utilization indicated the proportion of fermentable sugar and FAN that yeast consumed. The germination time of the rice malt improved the utilization of reducing sugar, FAN, and barley malt supplementation. Beer from 50% and 5^{th} days of germinated rice malt showed the maximum of FAN and reducing sugar utilization approximately 75% and 70%, respectively. At the same time, the beer from 90% and the 5^{th} days of germinated rice malt demonstrated only 50% and 57% of FAN and reducing sugar utilization, respectively. The incomplete of mashing process and inadequate of enzymes addition causing excessive amount of reducing sugar, FAN, dextrin, oligosaccharide, and non-fermentable sugar as well as protein in fermented wort.

Table 4.8 Percentage of FAN and reducing sugar utilization of fermented wort.

Treatments	**% Utilization**	
	FAN	**Reducing sugars**
1-50	61.50	60.06
1-70	64.10	56.73
3-50	65.03	62.21
3-70	68.95	57.61
5-50	75.16	68.99
5-70	69.67	62.43
5-90	49.56	57.21

4.6 Pilot scale brewing and qualities of final beer

4.6.1 Chemical properties and volatile compounds of final beer

Maltose was remained in final beer in the range of 2.30 – 4.60 g/L, while the inefficiency of maltotriose utilization was occurred in the 50 liters brewer, which was the cause of residual maltotriose in final beer product (Table 4.9). The amount of ethanol in beer was lower than standard commercial beer because of the lack of maltotriose consumed then converted to ethanol. The maximum ethanol concentration appeared in 50% of malt barley addition at approximately 4.00% (v/v), which was lower than standard commercial beer at 5.00%v/v (Table 4.10). Quantity of rice malt ratios had an effect on both pH and color of final beer. High ratio of rice malt influenced on the higher pH value and higher color value of final beer due to the non-enzymatic browning or Maillard reaction of rice husk during the mashing and wort boiling steps. The color of beer of 50% of rice malt was approximately 20 EBC units. The color of beer from 70% and 90% of rice malt was 30 – 32 EBC units in range of standard dark lager beer (20 – 50 EBC units).

มหาวิทยาลัยเทคโนโลยีสุรนารี

Table 4.9 Various types of fermentable sugar at the initial and finish fermentation.

Batch	Sugar concentration before fermentation (g/L)				Sugar concentration after fermentation (g/L)			
	Glucose	Fructose	Maltose	Maltotriose	Glucose	Fructose	Maltose	Maltotriose
1-50	17.87	2.42	31.72	18.08	0	0	2.62	17.13
1-70	16.74	1.11	27.45	19.91	0	0	2.32	17.34
3-50	21.75	2.39	31.66	17.92	0	0	3.30	17.54
3-70	19.34	1.73	22.28	14.99	0	0	2.90	17.40
5-50	26.79	3.27	33.97	17.49	0	0	4.63	15.93
5-70	20.55	1.73	22.89	14.15	0	0	3.93	13.29
5-90	21.34	1.83	19.02	13.00	0	0	2.59	12.96

Table 4.10 pH value, amount of ethanol and color of finished beer.

Batch	pH	Ethanol (%v/v)	Color (EBC)
1-50	4.84	3.66^{d}	20.6^{a}
1-70	5.88	3.46^{b}	31.6^{b}
3-50	4.74	4.07^{e}	21.9^{a}
3-70	5.81	3.67^{d}	32.9^{c}
5-50	5.00	3.97^{e}	20.3^{a}
5-70	5.95	3.55^{c}	29.2^{b}
5-90	6.06	3.18^{a}	30.5^{b}

Significant $P<0.05$ in the same column

Volatile compounds including higher alcohol or fusel alcohol, estery compounds, and free fatty acid in final beer were determined as summarized in Table 4.10. Isoamyl alcohol or 3-methyl-1-butanol was in range of 570 – 700 ppm, which was higher than standard beer (60 ppm) and isobutanol in final beer was 9 – 29 ppm, which in range of isobutanol of standard beer (9.6 ppm). The isoamyl alcohol in final beer was high because of the intermediate substances of the isoamyl alcohol formation including leucine and 2-phenylalcohol was found in large amount. Biosynthesis of the isoamyl alcohol and the isobutanol was showed in figure 4.8. Calderbank and Hammond (1994) studied the influence of higher alcohol including the isoamyl alcohol and the isobutanol availability on ester formation. It was found that, the maximal rates of isoamyl acetate production occurred in 40 hours of fermentation that slightly later than the time of alcohol acetyl transferase (AAT) activity, which is the acetate ester synthesis enzyme. The isoamyl acetate formation occurs especially in the high gravity fermented wort (18°P) and was relatively effect by the availability of the isoamyl acetate. The addition of 400 mg/L of the isobutanol enhanced the rate of isoamyl acetate production during the fermentation in the normal gravity fermented wort (9.5°P). However, the formation of other estery compounds including ethyl acetate, isobutyl acetate and ethyl hexanoate was unaffected.

High level of rice malt and longer germination time improved the formation of estery compounds in final beer including isoamyl acetate, ethyl octanoate, and ethyl decanoate. It was found that, amount of ethyl octanoate on 70% of rice malt beer were 1.3, 7.8, and 9.9 ppm and ethyl decanoate was 2.9, 9.1, and 13.0 ppm on 1st, 3rd, and 5th days of germinated rice, respectively. In addition, the free fatty acids in beers were founds in abundance content (Table 4.11). As a result, octanoic acid and

hexanoic acid were in range of 138.3 – 602.4 ppm and 17.9 – 54.3 ppm, respectively. For the reason, the rice malts contain the high level of fat (approximately 3.0%), which higher than fat from malt barley. Volatile fatty acid from the lipase during mashing step, the precursors of estery compounds increased when longer germinated time and higher level of rice malt was used. However, the residual of volatile fatty acid in final beer was the main cause of the off-flavor in beer. Šmogrovičová and Dömény (1999) reported that the present of 5 ppm of the hexanoic acid and 10 ppm of the octanoic acid were characterized the cheesy, goaty, and sweaty flavor. Furthermore, Bosswell *et al.*, (2002) reported that the mechanical agitation during the fermentation of beer improved the some estery compounds and fusel alcohol including isoamyl acetate, isobutyl acetate, isobutanol, and isoamyl acetate, but reduced the formation of ethyl acetate and ethyl hexanoate.

Table 4.11 Volatile compounds of final beer.

Compounds	**Concentrations (ppm)**						
	1-50	**3-50**	**5-50**	**1-70**	**3-70**	**5-70**	**5-90**
Isoamyl alcohol	674.9^{bc}	709.5^{c}	585.2^{ab}	648.4^{abc}	603.4^{ab}	619.1^{abc}	571.2^{a}
Isobutanol	28.4^{c}	19.9^{bc}	17.7^{ab}	26.8^{bc}	25.8^{bc}	21.1^{bc}	8.9^{a}
Isoamyl acetate	15.7^{a}	22.3^{ab}	18.6^{a}	20.3^{ab}	6.0^{b}	41.8^{d}	33.6^{c}
Ethyl octanoate	3.6^{ab}	2.0^{a}	4.0^{ab}	1.3^{a}	7.8^{ab}	9.9^{b}	10.5^{b}
Ethyl decanoate	7.1^{abc}	5.0^{ab}	6.9^{abc}	2.9^{a}	9.1^{abc}	3.0^{c}	12.5^{bc}
Octanoic acid	244.0^{b}	268.9^{b}	364.6^{c}	138.3^{a}	431.7^{d}	468.6^{d}	602.4^{e}
Hexanoic acid	$41.2b^{c}$	41.3^{bc}	52.8^{d}	17.9^{a}	34.8^{b}	48.1^{cd}	54.3^{d}

Significant $P<0.05$ in the same row

4.6.2 Sensory evaluation of final beer

The sensory analysis test was completed by 12 assessors at SUT. There were five attributes required to evaluate including appearance, aroma, flavor, mouth-feel, and overall impression. The appearance of beer was evaluated according to sight, color, and clearance of beer. At 50% rice malt addition in difference germination time had average score at 3.0 (like) as well as beer from the 5^{th} days of germinated rice at difference barley ratios was non-significantly different. However, 70% of rice malt ratio at the 1^{st} and 3^{rd} day of germinated rice (1-70 and 3-70 beer) had the average appearance score at 1.50 (dislike). Moreover, 70% rice malt ratio for 1^{st} and 3^{rd} day of germination was found the excessive amount of non-saccharification starch and dextrin, which caused the opaque appearance in beer.

Figure 4.9A showed the sensory evaluation score of 50% of rice malt addition at difference germinated time. Flavor and mouth-feel from three treatments (1-50, 3-50, and 5-50) of beer was found non-significantly different with scored at 3.0 and 3.2, respectively. Its indicated that the flavor and mouth-feels of beer brewed from 50% rice malt was more favorable. Figure 4.9B showed the sensory evaluation score of 70% of rice malt addition at difference germinated time. The score of all attributes was lower than that 50% rice malt beer. Particularly, the beer from the 1^{st} day of germination (1-70 beer) had the low evaluation score as 1.0 for flavor, aroma, and overall impression, therefore it was specified as undrinkable. According the result, the assessors disliked beer from the 1^{st} day of germination because it contained high ratio of non-modify rice grains and contributed the sweet flavor and aroma which was extremely non-acceptable properties in this beer. While the evaluation score of the 3-70 and 5-70 beer was similar excepted the appearance property which was lower than from

50% rice malt beer. The overall impression score of beer was 2.0, which indicated the overall acceptance was normal but drinkable. In summary, the qualities of rice malt unaffected on qualities of beer when the 50% of barley malt was supplemented. Conversely, when increased the ratios of rice malt to 70%, the qualities of rice malt express as the germinated time of rice was influenced. For example, the modification, gelatinization properties, and enzymes formation during the germination of grains or the amount of FAN was affected. The acceptability of beer was direct variation on quantity of barley malt addition and qualities of rice malt.

The deficiency of flavor and aroma as well as overall acceptance score of beer from rice malt based consistent with result of volatile compounds (Table 4.10) in beer, which contained the high level of volatile fatty acid such as octanoic acid, decanoic acid, and hexanoic acid that contributed the rancidity of beer. Fusel alcohol in beer, especially isoamyl alcohol was approximately ten times higher than standard and threshold. Finally, the sweetness that consequence on aftertaste of beer from the existing of non-fermentable sugar, for example maltotetraose, maltopentaose, dextrin, and oligosaccharide and particularly maltotriose, due to the insufficiency of amylolytic enzymes particularly β-amylase also mashing time or reaction time.

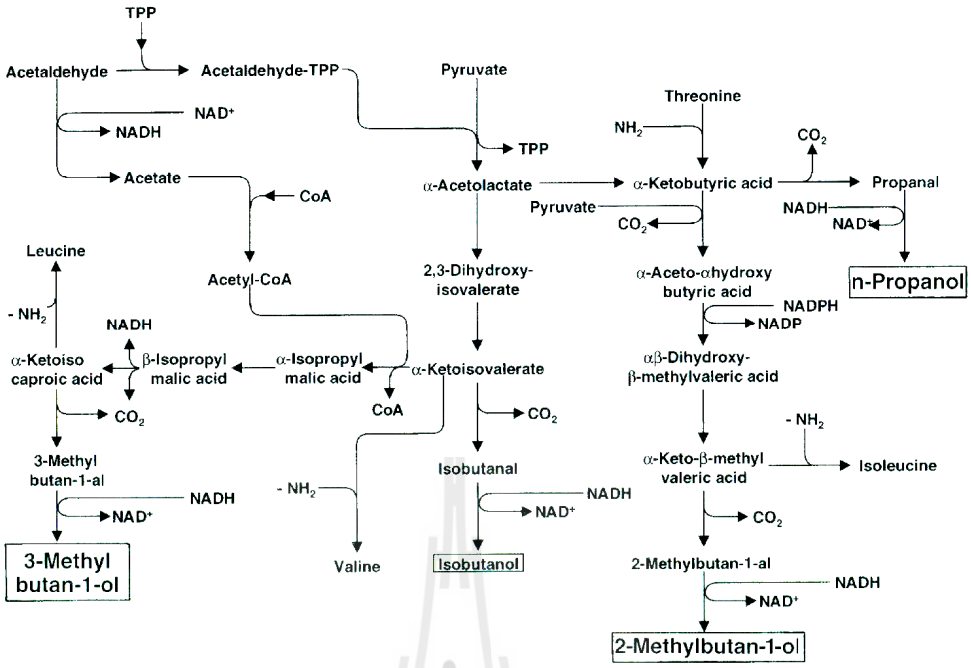


Figure 4.9 Biosynthesis pathways for synthesis of some higher alcohols.

Source: Boulton and Quain (2001)

มหาวิทยาลัยเทคโนโลยีสุรนารี

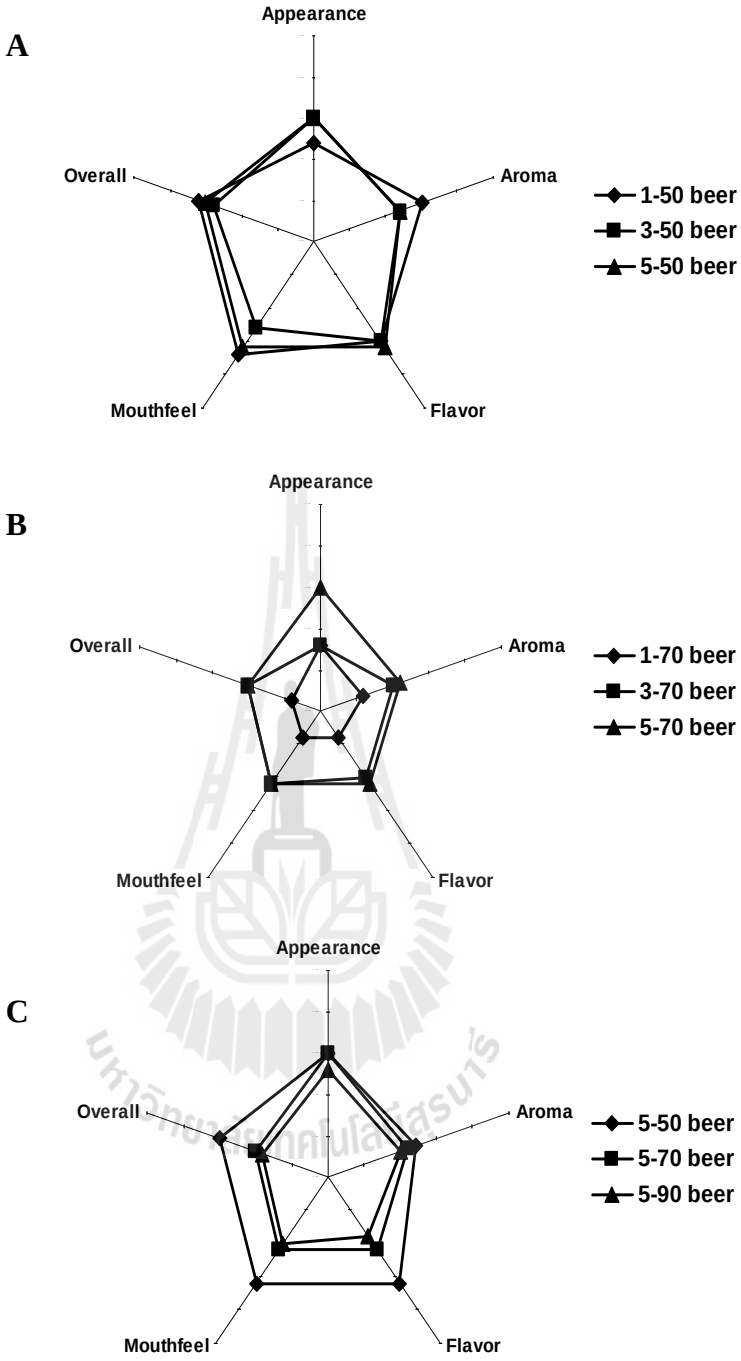


Figure 4.10 Spider web diagram of the sensory result of beer tasting

(A) 50% of rice malt at difference germination time

(B) 70% of rice malt at difference germination time

(C) 5th days of germinated rice at difference rice malt ratios

CHAPTER V

CONCLUSIONS

The influences of germination time on the rice malt qualities including malting loss, FAN, extract content of malt and enzymes activities including α- amylase and β-amylase were carried out. Extract content and FAN constantly increased during the third to ninth days of germination. The maximum extract content occurred in ninth days of germination but the extract content of rice was not reached the typical brewing malt (80%). Ninth day of germinated malt provided 145 mg/100 g malt of FAN, which non-significantly difference with FAN in malt barley (150 mg/100 g malt). Whereas, the quality of germinated rice malt in the 1^{st} day was non-significantly different with unmalt rice (control). Rice malt from the 7^{th} and 9^{th} days of germination was negligible because more than 20% malting loss. Commercial enzymes including heat stable α-amylase, bacterial protease and malt barley were supplemented for improving the qualities of wort using RSM technique. The suitability of model was analyzed and expressed as *p*-value. Germination time of rice, commercial α-amylase and barley addition affected on extract content, yield, and fermentable sugar in wort. Whereas, the germination time of rice and bacterial protease affected on the FAN in wort. However, the protease in germinated malt higher impact to the FAN than commercial enzyme addition. The appropriate conditions for mashing condition based on germination time and rice malt ratios were determined. The rice malt from the 5^{th} days of germination

showed the maximum rice malt ratio at 90% with 0.40 g/100g malt of both enzymes supplementation.

Total fermentable sugars of wort from the 5th days of germination at 50% were non-significantly difference with standard wort at the same concentration. However, the amount of maltose in standard wort was higher than experimental wort roughly 3 times, whereas the glucose from experimental wort was quite high about 33% of total fermentable sugar. The germination time of rice malt and barley malt addition improved both reducing sugar and FAN consumption. Nevertheless, the highest utilization of reducing sugar from the experiment was 70%, which was lower than wort from barley malt at more than 80% utilization. The lack of maltotriose consumption occurred in every treatments, which is the main cause of low ethanol production. Amount of rice malt ratio was influenced on the color of beer due to the Maillard reaction of rice husk during mashing and wort boiling.

Isoamyl alcohol in experimental beer was 570 – 700 ppm, which was higher than that of the standard beer. The rice malt ratio and the germination time improved the formation of estery compounds in final beer including isoamyl acetate, ethyl octanoate, and ethyl decanoate. Qualities of rice malt had no effect on sensory score when the 50% of barley malt was supplemented. Conversely, the ratio of rice malt to 70% influenced the qualities of rice malt. Twelve panelists was judged the beer from 50% of rice malt as good while, beer from 70% rice malt was judged as normal and drinkable.

REFERENCES

Agu, R. C. and Palmer, G. H. (1997). Effect of mashing procedures on some sorghum varieties germinated at different temperature. **Process Biochemistry**. 32(2): 147-158.

Agu, R. C. and Palmer, G. H. (1998). A reassessment of sorghum for lager beer brewing. **Bioresource Technology**. 66: 253-261.

Ahmad, A. L., Wong, S. S., Teng, T. T. and Zuhairi, A. (2007). Optimization of coagulation-flocculation process for pulp and paper mill effluent by response surface methodological analysis. **Journal of Hazardous Materials**. 145: 162-168.

Alves, L. S., Herberts, R. A., Hollatz, C., Miletti, L. and Stambuk, U. B. (2007). Maltose and maltotriose active transport and fermentation by *saccharomyces cerevisiae*. **Journal of American Society of Brewing Chemist**. 65(2): 99-104.

Anderson, R. G. and Kirsop, B. M. (1974). The control of volatile ester synthesis during the fermentation of wort of high specific gravity. **Journal of the Institute of Brewing**. 80; 48-55.

Ayraapaa, T. and Linstrom, I. (1973). Influence of long chain fatty-acid on the formation of esters by brewer's yeast. Proceedings of the 14th European Brewery Convention Congress. Quoted in Bamforth, C. W. (eds.). **Brewing: New Technology**. Woodhead publishing, CRC press.

Bao, J. and Bergman, C. J. (2001). The functionality of rice starch. Quote in Eliasson (eds.). **Starch in food: structure, function and applications**. Cambridge: Woodhead Publishing Limited.

Bamforth, C. W. (2009). **Handbook of Alcoholic Beverage Series: Beer A Quality Perspective**. Academic Press.

Boswell, C. D., Nienow, W. A. and Hewitt, C. J. (2002). Studies the effect of mechanical agitation on the performance of brewing fermentation: fermentation rate, yeast physiology and development of flavor compound. **Journal of American Society of Brewing Chemist**. 60(3): 101-106.

Boulton, C. and Quain, D. (2001). **Brewing yeast and fermentation.** Blackwell Science Ltd, Blackwell Publishing Company.

Bradee, L. H. (1977). Adjunct. The practical brewer. Quoted in Bamforth, C. W. (eds.). **Brewing: New Technology**. Woodhead publishing, CRC press.

Briggs, D. E. (1998). Malts and Malting. 1st ed. Blackie Academic & Professional, Chapman & Hall.

Briggs, D. E., Hough, J. S., Stevens, R. and Young, T. W. (1981). Malting and Brewing Science. Quoted in Priest, F. G. and Stewart, G. G. (eds.). **Handbook of brewing**. 2nd ed. CRC press.

Calderbank, J. and Hammond, J. R. M. (1994). Influence of higher alcohol availability on ester formation by yeast. **Journal of American Society of Brewing Chemist**. 52(2): 84-90.

Capanzana, M. V. and Buckle, K. A. (1997). Optimisation of Germination Conditions by Response Surface Methodology of a High Amylose Rice (*Oryza sativa*) Cultivar. **LWT**. 30(2): 155-163.

Carley, K. M., Kamneva, N. Y. and Reminga, J. (2004). **Response Surface Methodology CASOS Technical Report.** Center for Computational Analysis of Social and Organizational Systems**,** Carnegie Mellon University**.**

Celus, I., Brijs, K. and Delcour, J. A. (2006). The effect of malting and mashing on barley protein extractability. **Journal of Cereal Science**. 44: 203-211.

Clapperton, J. F. (1971). Simple peptides of wort and beer. **Journal of the Institute of Brewing**. 77: 177-180.

Comrie, A. A. D. (1967). Brewing liquor – a review. **Journal of the Institute of Brewing**. 73: 335-341. Quoted in Taylor, D.G. (2006). Water. In: Priest, F.G. and Stewart, G.G. (eds.). **Handbook of brewing**. 2nd ed. CRC press.

Coors, J. (1976). Practical experience with difference adjunct. **Technical Quarterly Master Brewers Association of the America**. 13; 117-123. Quote in Stewart, G. G. (2006). Adjuncts. Water. In: Priest, F.G. and Stewart, G.G. (eds.). **Handbook of brewing**. 2nd ed. CRC press.

Denault, L. J., Glenister, P. R. and Chau, S. (1981). Enzymology of the mashing step during beer production. **Journal of American Society of Brewing Chemists**. 39(2): 46-52.

Depraetere, F. A., Delvaux, F., Schutter, D. D.,Williams, I. S., Winderickx, J. and Delvaux, F. R. (2008). The influence of wort aeration and yeast preoxygenation on beer staling processes. **Food Chemistry**. 107: 242-249.

Eaton, B. (2006). An overview of brewing. In : Priest, F.G. and Stewart, G.G. (eds.). **Handbook of brewing**. 2nd ed. Taylor&Francis Group, CRC press.

Enari, T. M. and Sapanen, T. (1986). Metabolisation of endospermal reserves during germination of barley – centenary review. **Journal of the Institute of Brewing**. 91: 148-150.

European Brewery Convention, EBC (1998). Analytica-EBC. Germany.

Evans, E. E. (2005). Assessing the impact of the level of diastatic power enzymes and their thermostability on the hydrolysis of starch during wort production to predict malt fermentability. **Journal of American Society of Brewing Chemists**. 63(4): 185-198.

Figard, H., Girardc, C., Mougin, F., Demougeot, C. and Berthelot, A. (2008) Effects of aqueous hop (*Humulus Lupulus L.*) extract on vascular reactivity in rats: Mechanisms and influence of gender and hormonal status. **Phytomedicine**. 15: 185-193.

Gamlatha, J., Aldreda, G. P. and Panozzob, F. J. (2008). Barley (1→3; 1→4)-β-glucan and arabinoxylan content are related to kernel hardness and water uptake. **Journal of Cereal Science.** 47: 365-371.

Giovanni, M. (1983). Response surface methodology and product optimization. **Food Technology.** 37(11): 41-45.

Goldammer, T. (2000). **The Brewer's Handbook**. Apex Publisher, U.S.A.

Goode, D. L. and Arendt, E. K. (2006). Developments in the supply of adjunct material for brewing. In Bamforth, C. W. (eds.). **Brewing: New Technology**. Woodhead publishing, CRC press.

Goode, D. L. and Halbert, C. (2003). Optimization of mashing conditions when mashing with unmalt sorghum and commercial enzymes. **Journal of American Society of Brewing Chemists**. 68(2): 69-78.

Gorinstein, S., Kitov, S., Sarel, S., Berman, O., Berliner, M., Popovich, G. and Vermus, Y. (1980). Changes in the chemical composition of beer during the brewing process as a result of added enzymes. **Journal of American Society of Brewing Chemists**. 38(1): 23-26.

Hall, A. J., Babish, J. G., Darland, D. K., Carroll, B. J., Konda, V. R., Lerman, R. H., Bland, J. S. and Tripp, M. L. (2008). Safety, efficacy and anti-inflammatory activity of rho *iso*-alpha-acids from hops. **Phytochemistry.** 69: 1534-1547.

Hansen, J. (1999). Inactivation of *MXR1* Abolishes Formation of Dimethyl Sulfite from Ditethyl Sulfoxide in *Saccharomyces cerevisiae*. **Apply Environmental Microbiology**. 65(9): 3915-3919.

Hettiarachchy, N. S., Ju, Z. Y., Siebenmorgen, T. and Sharp, R. N. (2000). Rice production, processing and utilization. In : Kulp, K. and Ponte Jr., J.G. (eds.) **Handbook of cereal science and technology**. 2nd ed. Marcel Dekker, Inc.

Hockett, E. A. (2000). Barley. In : Kulp, K. and Ponte Jr., J.G. (eds.) **Handbook of cereal science and technology**. 2nd ed. Marcel Dekker, Inc.

Hoekstra, S. F. (1974). Fermentable sugars in wort, brew with adjuncts and fermented bottom yeast. Quote in Boulton, C. and Quain, D. (2006). **Brewing yeast and fermentation**. Blackwell Publishing.

Huey, B. A., Moldenhauer, K. K. and Helms, R. S. (1988). Rice varieties in Arkansas. University of Arkansas Extension Service.

Hughes, P. (2009). Beer Flavor. In : Bamforth, C. W. (eds.). Beer : A quality perspective. Academic Press.

Igyor, M. A., Ogbonna, A. C. and Palmer, G. H. (2001). Effect of malting temperature and mashing methods on sorghum wort composition and beer flavor. **Process Biochemistry**. 36: 1039-1044.

Ingledew, W. M. (1975). Utilization of wort carbohydrate and nitrogen by *Saccharomyces carlsbergensis*. Technical Quarterly of the Master Brewers Association of the Americas. 12; 145-50.

Iwuoha, C. I. and Aina, J. O. (1997). Effects of steeping condition and germination time on the alpha-amylase activity, phenolics content and malting loss of Nigerian local red and hybrid short Kaura sorghum malts. **Food Chemistry**. 58(4): 289 – 295.

Keßler, M., Zarnkow, M., Kreisz, S. and Back, W. (2005). Gelatinisation properties of different cereals and pseudocereals. **Mschr. Brauwissenschaft** 9/10: 82-88.

Kendall, N. T. (1995). Barley and malt. In : Hardwick, W. A. (eds). **Handbook of Brewing**. Marcel Dekker, Inc.

Kunze, W. (2004). Technology Brewing and Malting. 3rd ed. VLB Berlin, Germany.

Larsona, A. E., Rosa, R. Y., Lee O. A., Price, S., Haas, G. J. and Johnson, E. A. (1996). Antimicrobial activity of hop extracts against *Listeria monocytogenes* in media and in food. **International Journal of Food Microbiology**. 33: 195-207.

Lekkas, C., Stewart, G. G., Hill, A. E., Taidi, B. and Hodgson, J. (2007). Elucidation of the role of nitrogenous wort components in yeast fermentation. **Journal of the Institute of Brewing**. 113(1): 3-8.

Lewis, J. M. and Young, W. T. (1995). Brewing. London: Chapman & Hall.

Lindeboom, N., Chang, P. R. and Tyler, R. T. (2004). Analytical, biochemical and physicochemical aspects of starch granule size, with emphasis on small granule starch: a review. Starch, 56; 89-99. Quoted in Bamforth, C. W. (eds.). **Brewing: New Technology**. Woodhead publishing, CRC press.

MacWilliam, I. C. (1968). Wort composition – a review. **Journal of the Institute of Brewing**.74: 38-54.

Markovic, I., Deponte, R., Mari, V. and Johanides, V. (1995). Enzymic hydrolysis of protein grains mashing during barley spent grains mashing. **Process Biochemistry**. 30(5): 411-419.

Miller, L. G. (1951). Use of dinitrosalicylic acid reagent for determination of reducing sugar. **Analytical Chemistry**. 31(3): 426-428.

Montanari, L., Floridi, S., Marconi, O., Tironzelli, M. And Fantozzi, P. (2005). Effect of mashing procedures on brewing. **European Food Research and Technology**. 221: 175-179.

Moonjai, N. (2005). Comparative study of experimental beers brewed from rice malt with some starchy adjuncts. 31st Congress on Science and Technology at Suranaree University of Technology.

Mussatto, I. S., Dragone, G. and Roberto, C. I. (2006). Brewer's spent grain: generation, characteristics and potential applications. **Journal of Cereal Science**. 43: 1-14.

Myers, R. H. and Montgomery, D. C. (2002). Response Surface Methodology; Process and Product Optimization Using Designed Experiment. 2nd ed. John Wiley & Sons Inc, Canada.

Natarajan, P., Katta, S., Andrei, I., Babu Rao Ambati, V., Leonida, M. and Haas, G. J. (2008). Positive antibacterial co-action between hop (Humulus lupulus)constituents and selected antibiotics. **Phytomedicine**. 15: 194-201.

Nirmala, M., Subba Rao, M. V. S. S. T. and Muralikrishna, G. (2000). Carbohydrates and their degrading enzymes from native and malted finger millet (Ragi, Eleusine coracana, Indaf-15). **Food Chemistry**. 69 : 175-180.

Novak, S., Zechner-Krpan, V. and Marić, V. (2004). Regulation of maltose transport and metabolism in *saccharomyces cerevisiae.* **Food Technology and Biotechnology**. 42(3): 213-218.

O'Connor-Cox, E. S. C. and Ingledew, W. M. (1989). Wort nitrogen sources-Their use by brewing yeast; A review. **Journal of American Society of Brewing Chemist**. 47: 102-108.

Odibo, F. J. C., Nwankwo, L. N. and Agu, R. C. (2002). Production of malt extract and beer from Nigerian sorghum varieties. **Process Biochemistry**. 37: 851-855.

Palmer, G. H., Etokakpan, O. U. and Igyor, M. A. (1989). Journal of Applied Microbiology and Biotechnology. 5 : 265. Quote in Briggs, D. E. (1998). Malts and Malting. 1st ed. Blackie Academic & Professional, Chapman & Hall.

Palmer, G. H. (2006). Barley and malt. In : Priest, F.G. and Stewart, G.G. (eds.). **Handbook of brewing**. 2nd ed. Taylor&Francis Group, CRC press.

Patel, G. B. and Ingledew, W. M. (1973). Trends in wort carbohydrate utilization. **Applied Microbiology**. 26(3); 349-353.

Pickerel, A. T. W. (1986). The influence of free alpha-amino nitrogen in sorghum beer fermentations. **Journal of the Institute of Brewing**. 92; 568-571.

Pierce, J. S. (1987). Adjunct and the effect on beer quality. Proceedings of the 19th European Brewery Convention Congress. Quoted in Bamforth, C. W. (eds.). **Brewing: New Technology**. Woodhead publishing, CRC press.

Perpẽte, P., Santos, G., Bodart, E. and Collin, S. (2005). Uptake of amino acid during beer production: The concept of a critical time value. **Journal of American Society of Brewing Chemist**. 63(1): 23-27.

Popa, O., Babeanu, N., Vamanu, A. and Vamanu, E. (2007). The utilization of the response surface methodology for the optimization of cultivation medium and growth parameters in the cultivation of the yeast strain *S. cerevisiae* 3.20 on ethanol. **African Journal of Biotechnology.** 6(23) : 2700-2707.

Powell, C. D., Quain, D. E. and Smart, K. A. (2003). The impact of brewing yeast cell age on fermentation performance, attenuation and flocculation. **FEMS Yeast Research**. 3: 149-157.

Roberts, T. R. and Wilson, J. H. (2005). Hops. In : Priest, F.G. and Stewart, G.G. (eds.). **Handbook of brewing**. 2nd ed. Taylor&Francis Group, CRC press.

Rodrigues, L., Teixeira, J., Oliveira, R. and Van der Mei, H. D. (2006). Response surface optimization of the medium components for the production of biosurfactants by probiotic bacteria. **Process Biochemistry**. 41: 1-10.

Rourke, T. O. (2003). Hops and hop product: Technical summary. **The Brewer International**. 3(1): 21-25.

Salubchua, J., Srakeaw, K. and Moonjai, N. (2005). Primary study of rice beer fermentation. 31st Congress on Science and Technology at Suranaree University of Technology.

Schwarz, P., Stanley, P. and Solberg, S. (2002). Activity of lipase during mashing. **Journal of the Institute of Brewing**. 60(3): 107-109.

Šmogrovičová, D. and Dömény, Z. (1999). Beer volatile by-product formation at different fermentation temperature using immobilized yeasts. **Process Biochemistry**. 34: 785-794.

Stevens, J. F. and Page, J. E. (2004). Xanthohumol and related prenylflavonoids from hops and beer: to your good health. **Phytochemistry**. 65: 1317-1330.

Taylor, D. G. (1981). How water composition affects the taste of beer. **Brewing and Distillation International.** 11: 35-37.

Taylor, D. G. (1990). The importance of pH control during brewing. Quoted in Taylor, D.G. (2006). Water. In: Priest, F.G. and Stewart, G.G. (eds.). **Handbook of brewing**. 2nd ed. CRC press.

Teng, J., Stubits, M. and Lin, E. (1983). The impotence of rice variety selection for optimum brewhouse operation. Proceedings of the 19th European Brewery Convention Congress. Quoted in Bamforth, C. W. (eds.). **Brewing: New Technology**. Woodhead publishing, CRC press.

Villar, J. C., Cañete, R. E. and Manganelly, E. A. (2004) Why adding rice hull ash can benefit beer clarification. **Filtration & Separation**. 41(6): 32-33.

Vohra, A. and Satyanarayana, T. (2002). Statistical optimization of the medium components by response surface methodology to enhance phytase production by *Pichia anomala*. **Process Biochemistry**. 37: 999-1004.

Wijngaard, H. H. and Aredt, E. K. (2006). Optimization of a mashing program for 100% malted buckwheat. **Journal of the Institute of Brewing**.112 (1): 57-65.

Wang, S., Chen, F., Wu, J., Wang, Z., Liao, X. and Hu, X. (2007). Optimization of pectin extraction assisted by microwave from apple pomace using response surface methodology. **Journal of Food Engineering**. 78: 693-700.

Zhao, J., F., Fortune, S., Barbosa, V., L., McGrath, S., P., Stobart, R., Bilsborrow, P., E., Booth, E., J., Brown, A. and Robson, P. (2006) Effects of sulphur on yield and malting quality of barley. **Journal of Cereal Science**. 43: 369-377.

Zheng, X., D'Amore, T., Russell, I. and Stewart, G. G. (1994). Factors influencing maltotriose utilization during brewery wort fermentations. **Journal of American Society of Brewing Chemist**. 52(2): 41-47.

Zhao, R., Zhang, Z., Zhang, R., Li, M., Lei, Z., Utsumi, M. and Sugiura, N. (2010). Methane production from rice straw pretreated by a mixture of acetic–propionic acid. **Bioresource Technology**. 101: 990-994.

Zhong, K. and Wang, Q. (2010). Optimization of ultrasonic extraction of polysaccharides from dried longan pulp using response surface methodology. **Carbohydrate Polymers**. 80: 19-25.

Beeradvocate (2008). Yeast guide. Available: http://beeradvocate.com/beer/101/yeast.

Botanix (2008). The versatility of hops. Available: www.botanix.co.uk.

Wikipedia (2008). Beer. Available: http://en.wikipedia.org/wiki/Beer.

Wikipedia (2008). Rice. Available: http://en.wikipedia.org/wiki/Rice.

Wisconsin-Madison Department of Bacteriology (2008). The diversity of metabolism in prokaryotes. Available: www.textbookofbacteriology.net/metabolism.

APPENDICES

APPENDIX A

ANALYSIS OF RESPONSE SURFACE

Table 1A Experimental design of response surface

Runs	Coded value				Actual value			
	X_1	X_2	X_3	X_4	Germination time (days)	Rice malt (%)	α-amylase (g/100g malt)	Protease (g/100g malt)
1	-1	-1	-1	1	1	50	0.00	0.50
2	0	1	0	0	3	100	0.25	0.25
3	0	0	1	0	3	75	0.50	0.25
4	1	-1	-1	1	5	50	0.00	0.50
5	1	1	-1	-1	5	100	0.00	0.00
6	1	1	1	-1	5	100	0.50	0.00
7	1	0	0	0	5	75	0.25	0.25
8	1	-1	1	-1	5	50	0.50	0.00
9	-1	-1	-1	-1	1	50	0.00	0.00
10	-1	-1	1	1	1	50	0.50	0.50
11	-1	1	-1	-1	1	100	0.00	0.00
12	0	0	0	-1	3	75	0.25	0.00
13	0	0	0	0	3	75	0.25	0.25
14	-1	0	0	0	1	75	0.25	0.25
15	-1	1	1	1	1	100	0.50	0.50
16	0	-1	0	0	3	50	0.25	0.25
17	-1	1	1	-1	1	100	0.50	0.00
18	-1	1	-1	1	1	100	0.00	0.50
19	0	0	0	1	3	75	0.25	0.50
20	1	1	-1	1	5	100	0.00	0.50
21	1	-1	-1	-1	5	50	0.00	0.00
22	-1	-1	1	-1	1	50	0.50	0.00
23	0	0	0	0	3	75	0.25	0.25
24	1	1	1	1	5	100	0.50	0.50
25	0	0	-1	0	3	75	0.00	0.25
26	1	-1	1	1	5	50	0.50	0.50

มหาวิทยาลัยเทคโนโลยีสุรนารี

Table 2A Responses value of each experimental design

Runs	Coded value				Responses value			
	X_1	X_2	X_3	X_4	Extract (%)	FAN (mg/L)	Yield (%)	Fermentable sugar (g/L)
1	-1	-1	-1	1	80.1	244	74.5	70.1
2	0	1	0	0	76.3	146	83.0	43.8
3	0	0	1	0	83.0	205	93.0	74.9
4	1	-1	-1	1	87.2	323	75.6	83.6
5	1	1	-1	-1	37.4	206	54.0	43.9
6	1	1	1	-1	76.6	201	83.6	66.7
7	1	0	0	0	87.1	322	87.4	74.4
8	1	-1	1	-1	88.3	245	85.3	97.7
9	-1	-1	-1	-1	84.0	153	77.6	81.0
10	-1	-1	1	1	90.6	259	86.8	96.3
11	-1	1	-1	-1	16.5	41	28.0	17.5
12	0	0	0	-1	87.0	145	87.1	68.7
13	0	0	0	0	84.1	217	84.6	64.3
14	-1	0	0	0	83.1	196	92.7	55.6
15	-1	1	1	1	76.4	140	89.6	48.6
16	0	-1	0	0	89.9	269	94.1	86.0
17	-1	1	1	-1	74.7	36	85.2	42.0
18	-1	1	-1	1	20.3	150	36.0	22.5
19	0	0	0	1	82.7	238	94.6	63.0
20	1	1	-1	1	79.5	323	77.2	46.1
21	1	-1	-1	-1	83.6	183	81.4	74.3
22	-1	-1	1	-1	81.9	146	87.0	87.3
23	0	0	0	0	82.7	203	87.1	69.8
24	1	1	1	1	79.5	256	86.3	61.4
25	0	0	-1	0	79.8	197	85.8	47.3
26	1	-1	1	1	91.2	309	105.5	78.9

APPENDIX B

STANDARD CURVES

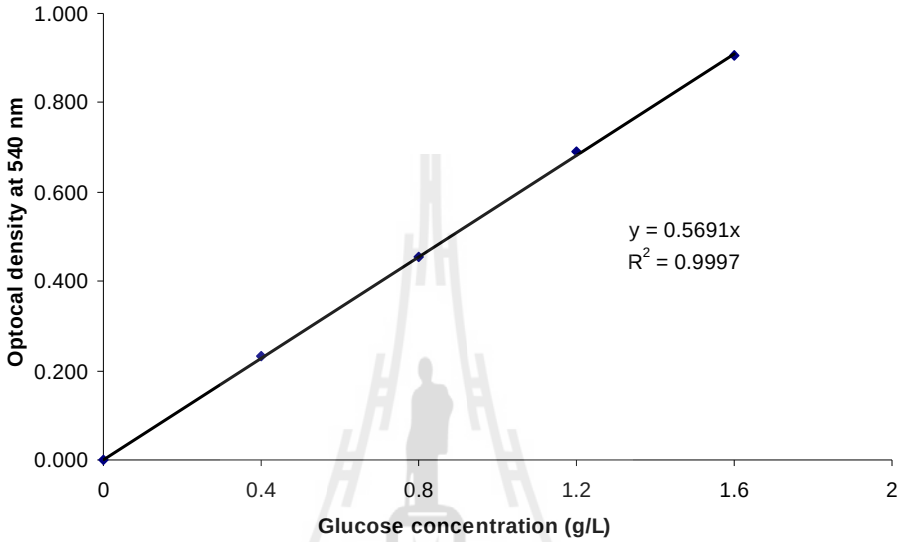


Figure 1B Standard curve of standard glucose using DNS method.

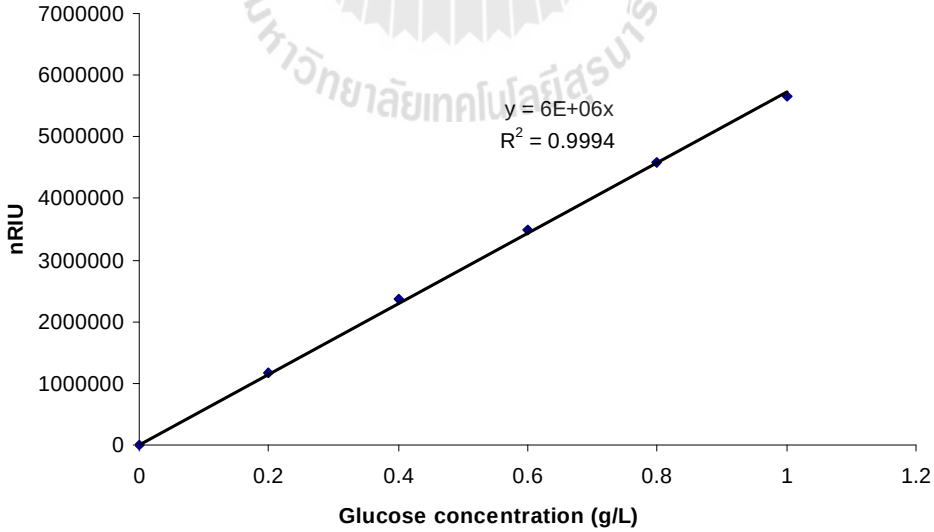


Figure 2B Standard curve of standard glucose using Vertisep^{TM} OA column with DI water mobile phase and RI detector.

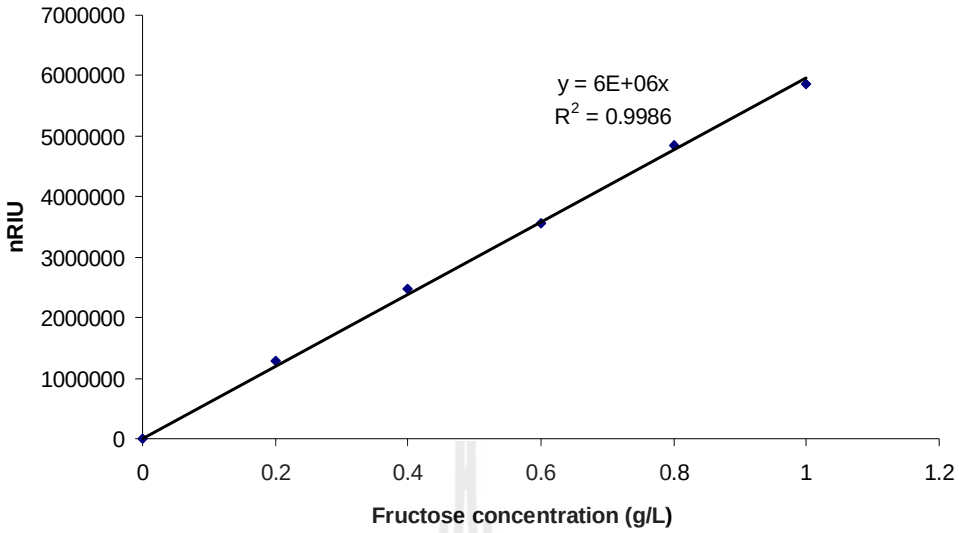


Figure 3B Standard curve of standard fructose using Vertisep^{TM} OA column with DI water mobile phase and RI detector.

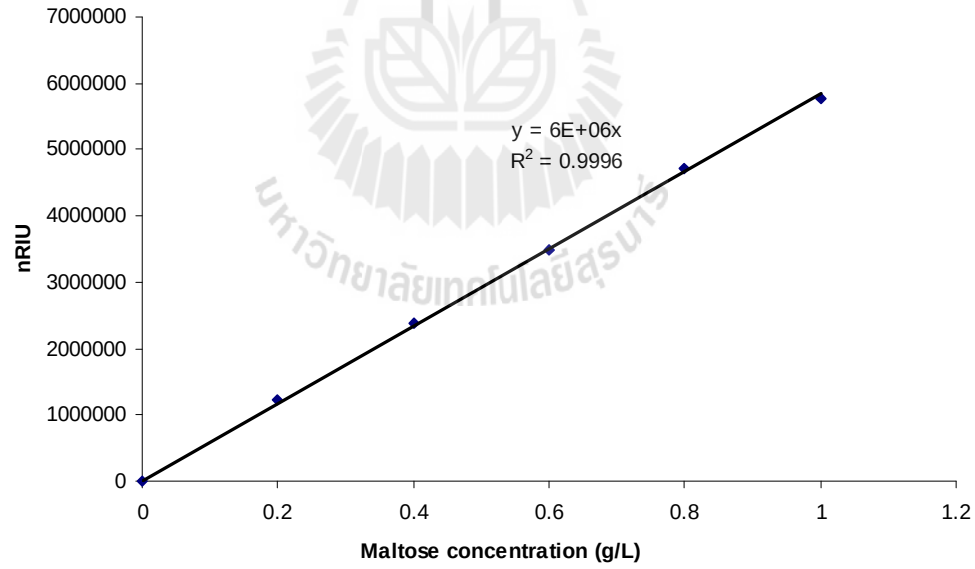


Figure 4B Standard curve of standard maltose using Vertisep^{TM} OA column with DI water mobile phase and RI detector.

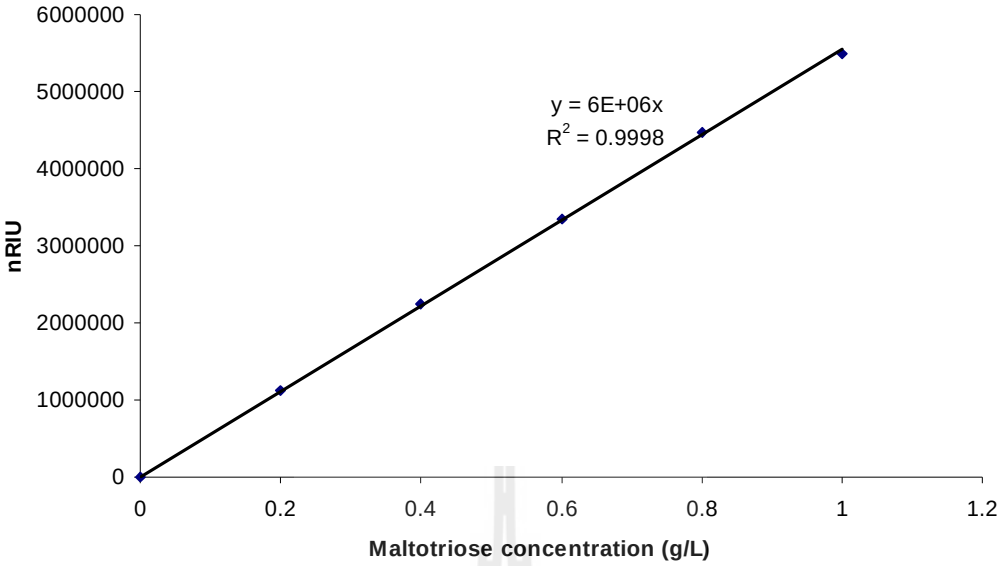


Figure 5B Standard curve of standard maltotriose using Vertisep^{TM} OA column with DI water mobile phase and RI detector.

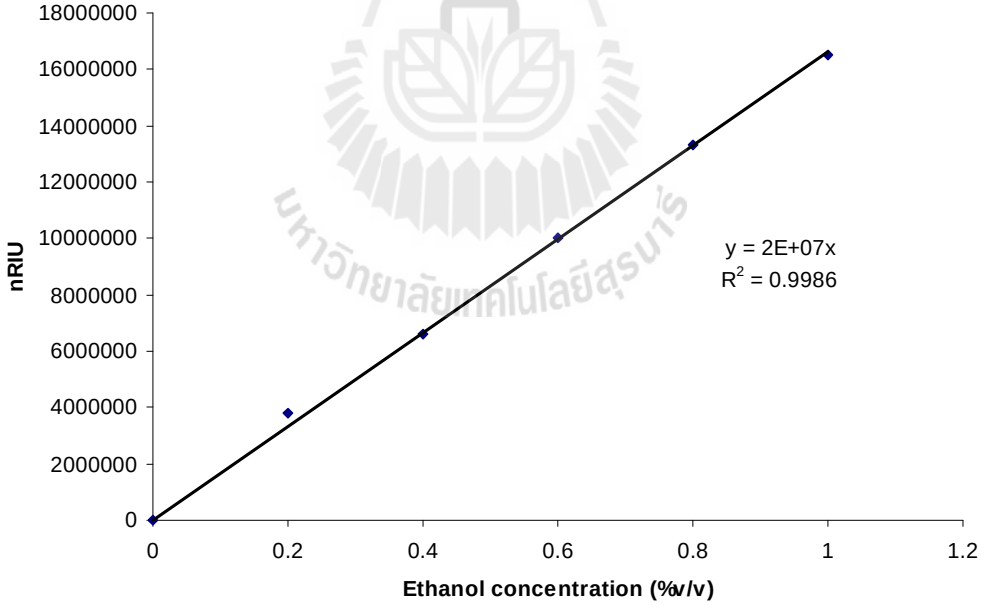


Figure 6B Standard curve of standard ethanol using Vertisep^{TM} OA column with DI water mobile phase and RI detector.

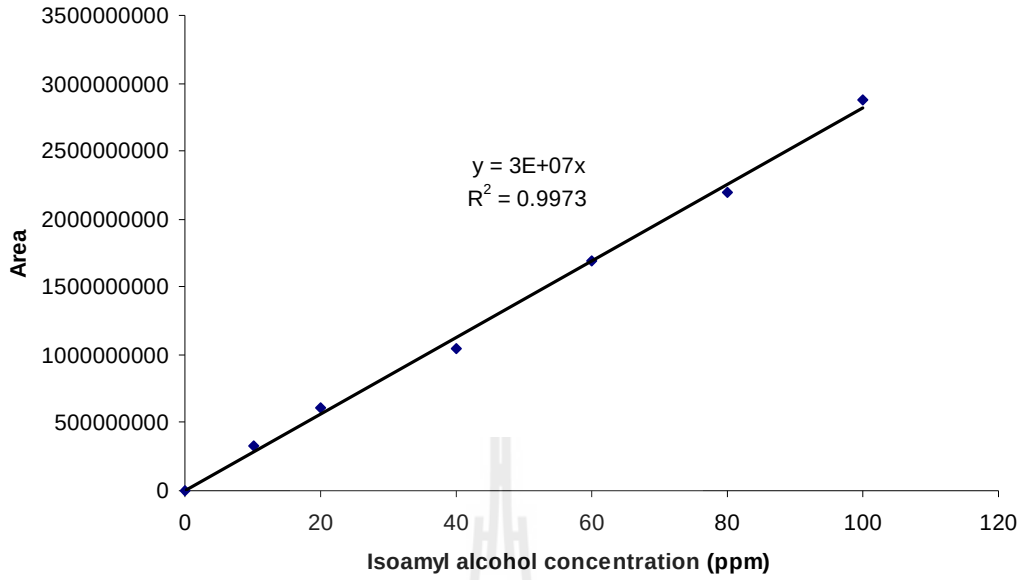


Figure 7B Standard curve of standard isoamyl alcohol by GC-MS using DB-WAX column with SPME technique.

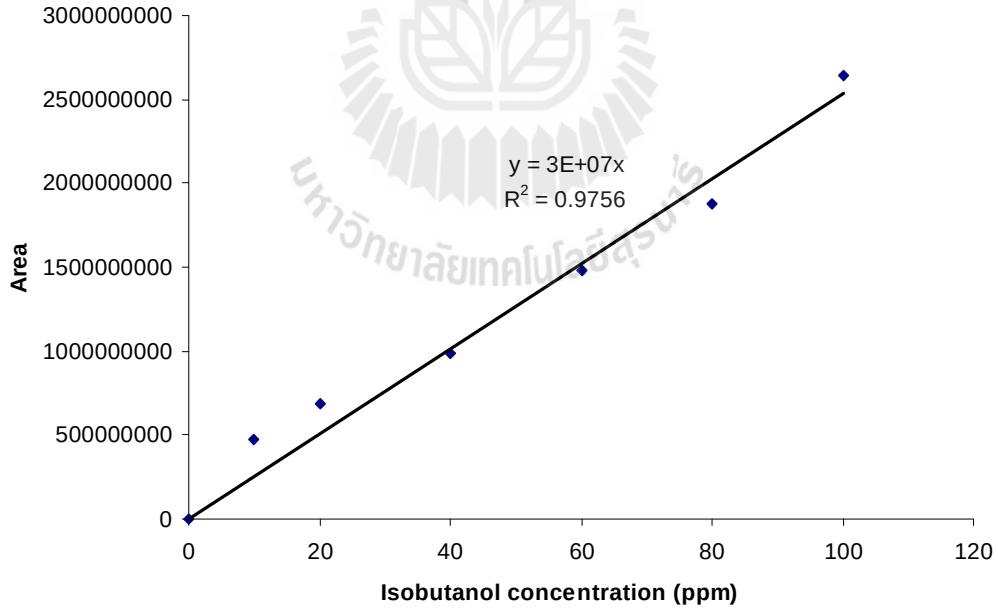


Figure 8B Standard curve of standard isobutanol by GC-MS using DB-WAX column with SPME technique.

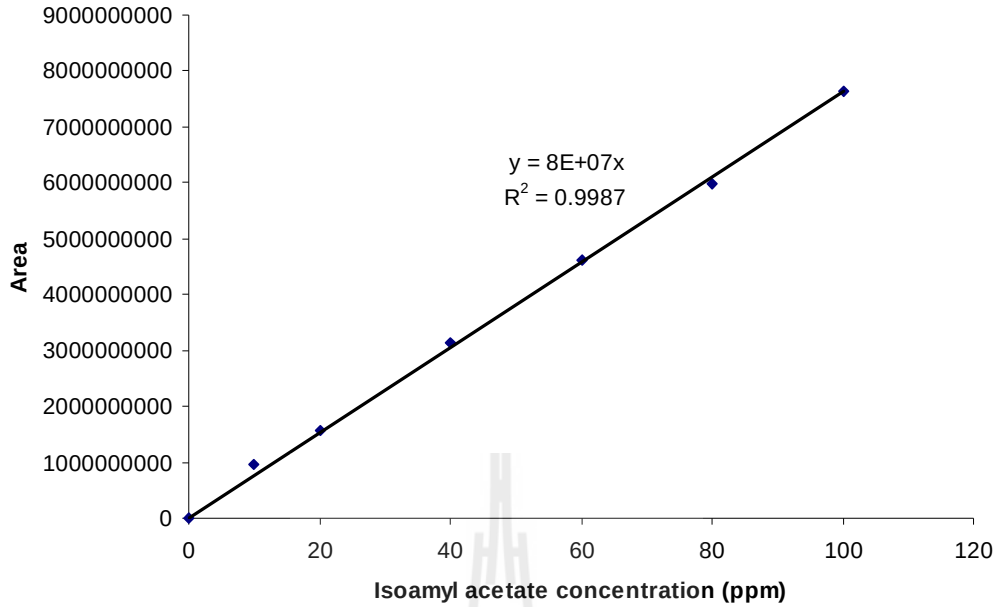


Figure 9B Standard curve of standard isoamyl acetate by GC-MS using DB-WAX column with SPME technique.

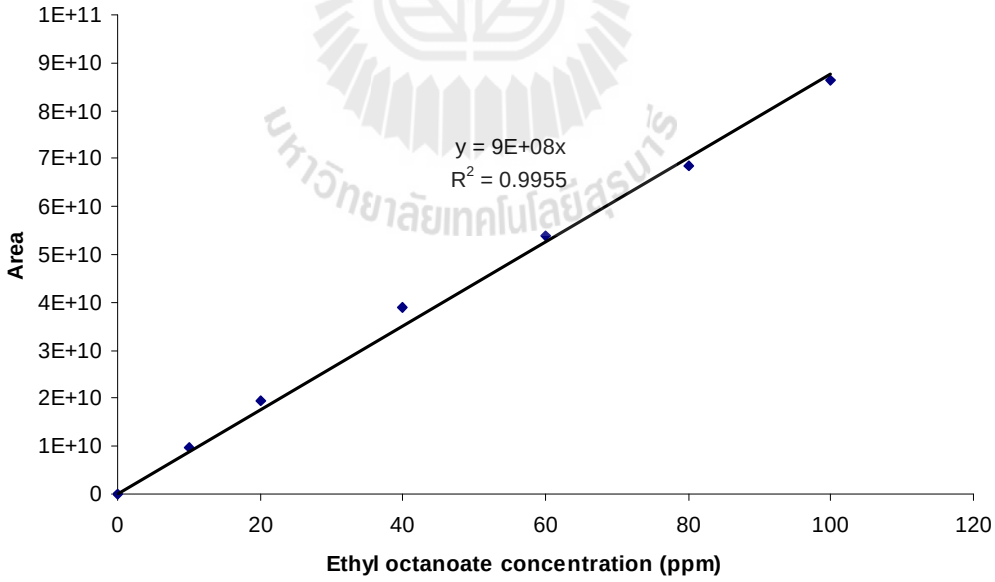


Figure 10B Standard curve of standard ethyl octanoate by GC-MS using DB-WAX column with SPME technique.

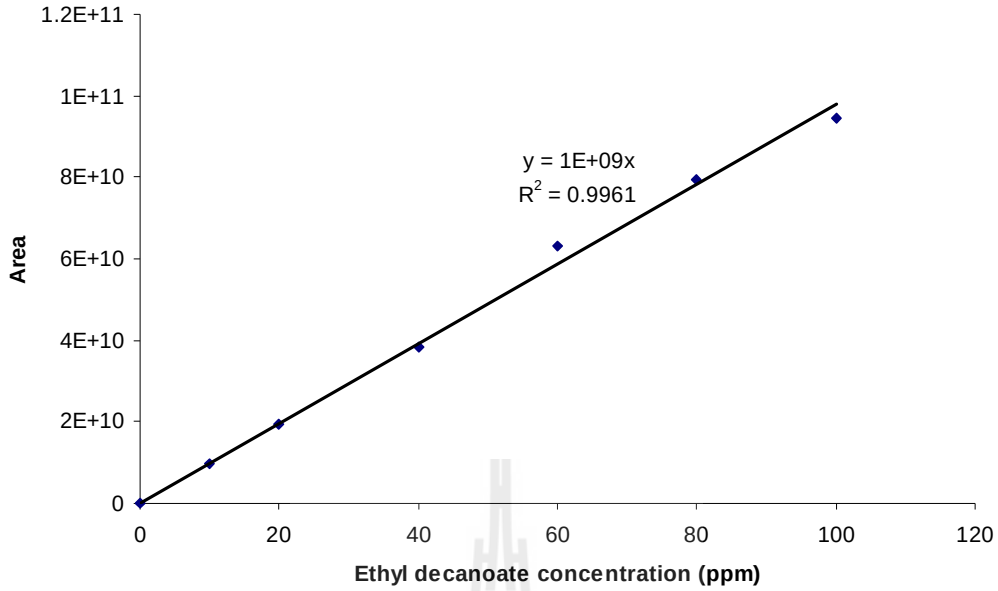


Figure 11B Standard curve of standard ethyl decanoate by GC-MS using DB-WAX column with SPME technique.

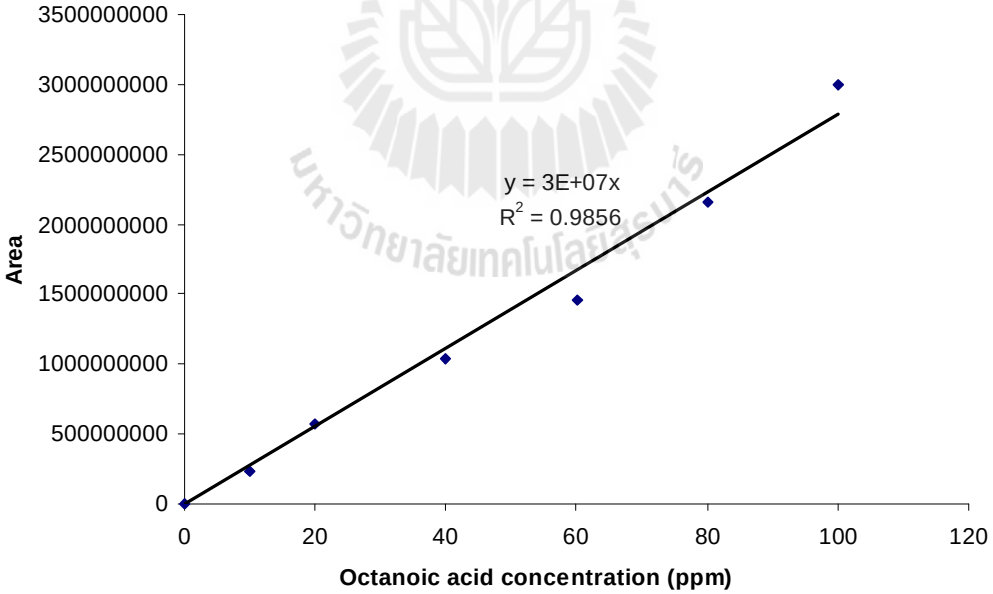


Figure 12B Standard curve of standard octanoic acid by GC-MS using DB-WAX column with SPME technique.

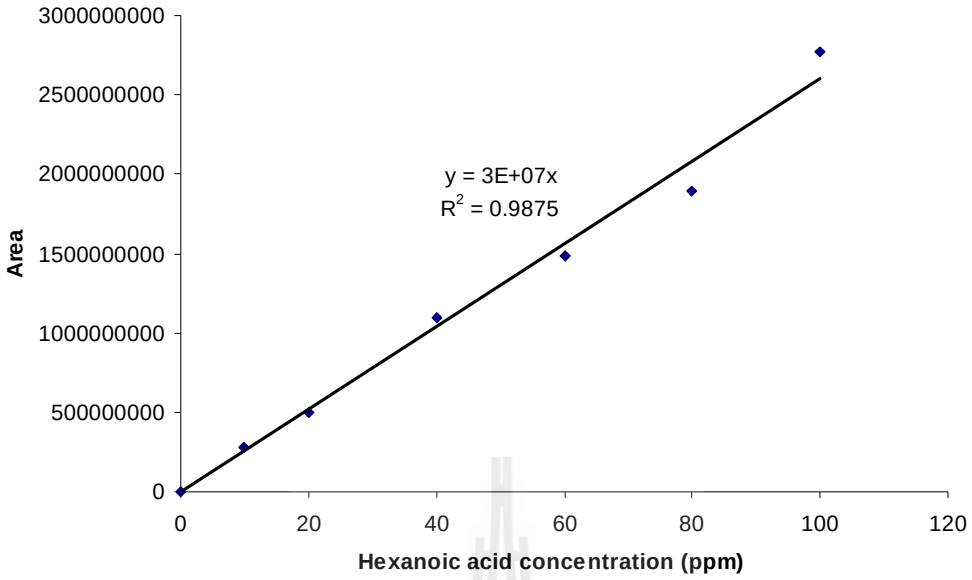


Figure 13B Standard curve of standard hexanoic acid by GC-MS using DB-WAX column with SPME technique.

มหาวิทยาลัยเทคโนโลยีสุรนารี

APPENDIX C

CHROMATOGRAMS

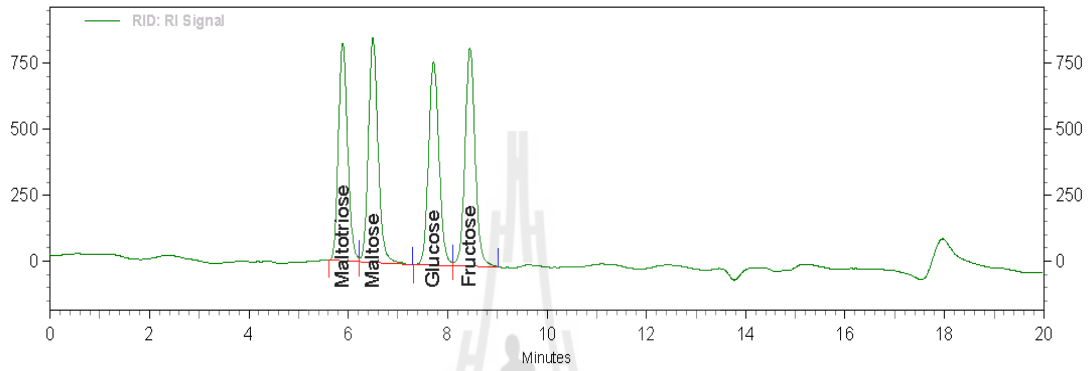


Figure 1C HPLC chromatogram of 0.2 g/L of each standards glucose, fructose, maltose and maltotriose using Vertisep^{TM} OA column with DI water mobile phase and RI detector.

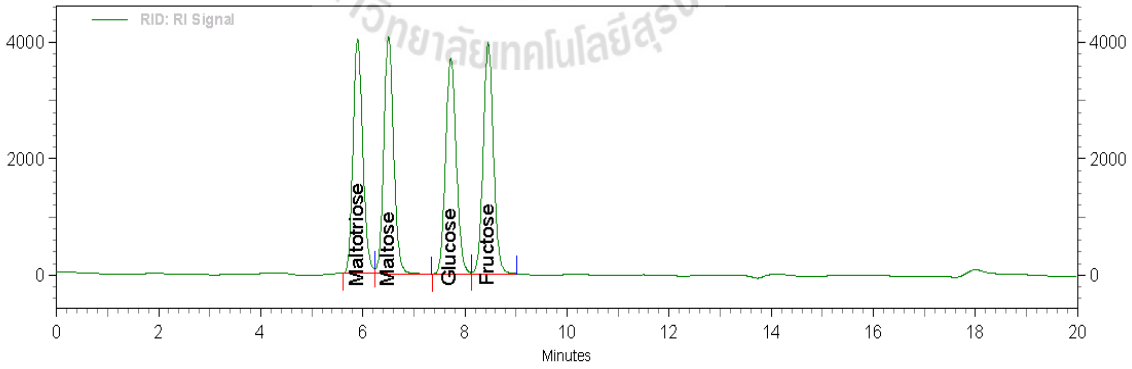


Figure 2C HPLC chromatogram of 1 g/L of each standards glucose, fructose, maltose and maltotriose using Vertisep^{TM} OA column with DI water mobile phase and RI detector.

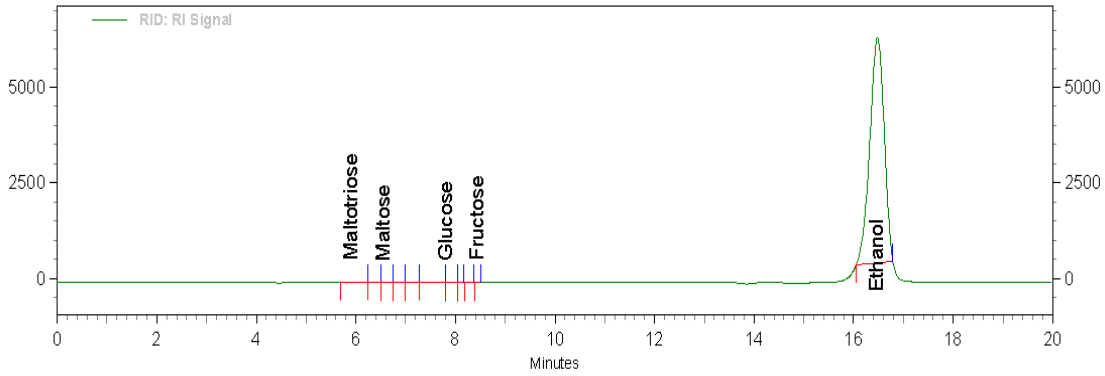


Figure 3C HPLC chromatogram of 0.8 g/L of standard ethanol using Vertisep^{TM} OA column with DI water mobile phase and RI detector.

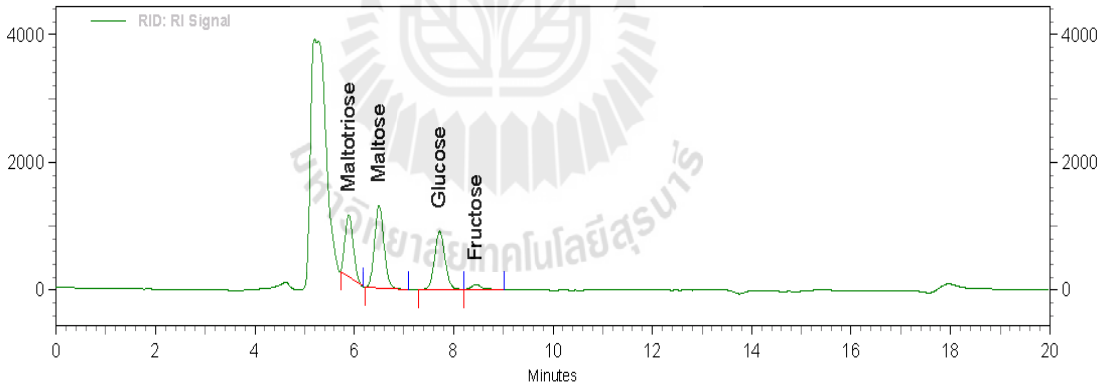


Figure 4C HPLC chromatogram of fermentable sugars in experimental wort sample 2 using Vertisep^{TM} OA column with DI water mobile phase and RI detector.

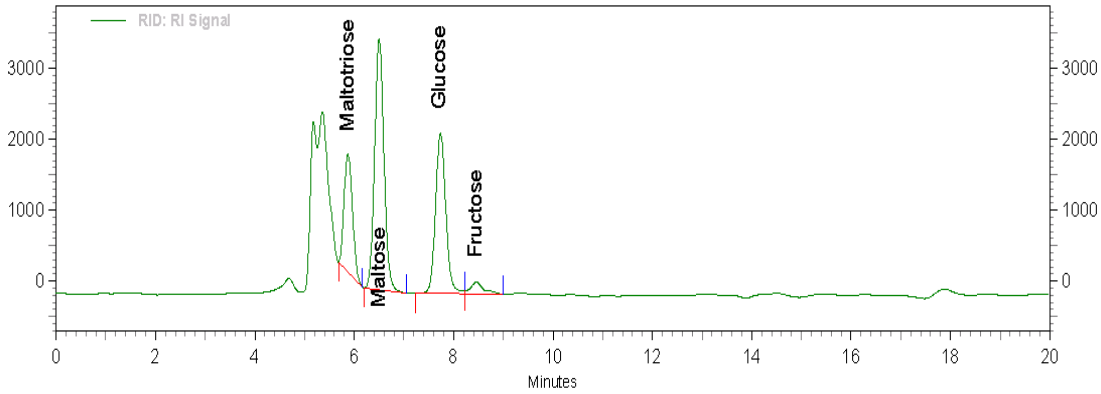


Figure 5C HPLC chromatogram of fermentable sugars in experimental wort sample 10 using Vertisep^{TM} OA column with DI water mobile phase and RI detector.

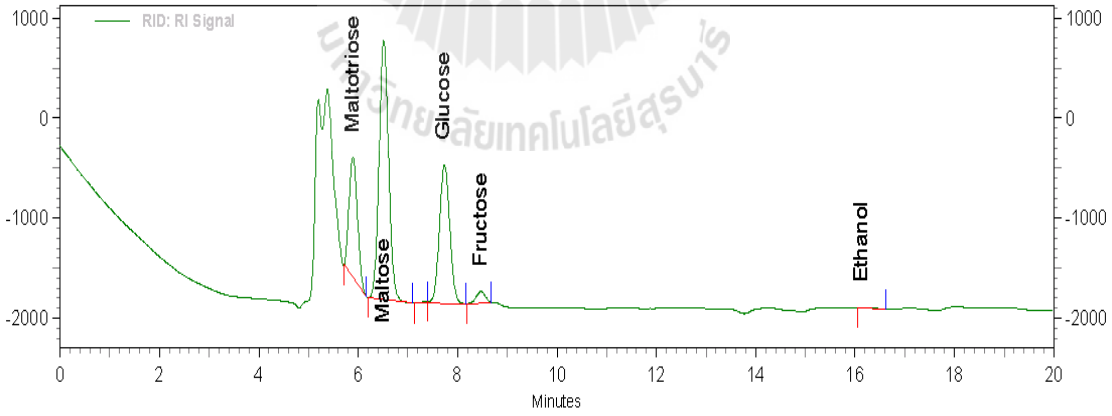


Figure 6C HPLC chromatogram of fermentable sugars in 1-50 wort at 0 h using Vertisep^{TM} OA column with DI water mobile phase and RI detector.

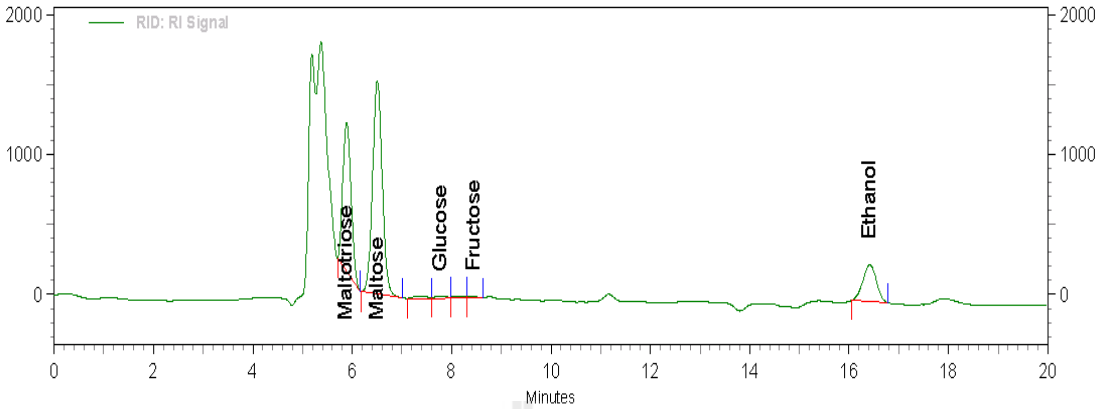


Figure 7C HPLC chromatogram of fermentable sugars in 1-50 wort at 48 h using Vertisep^{TM} OA column with DI water mobile phase and RI detector.

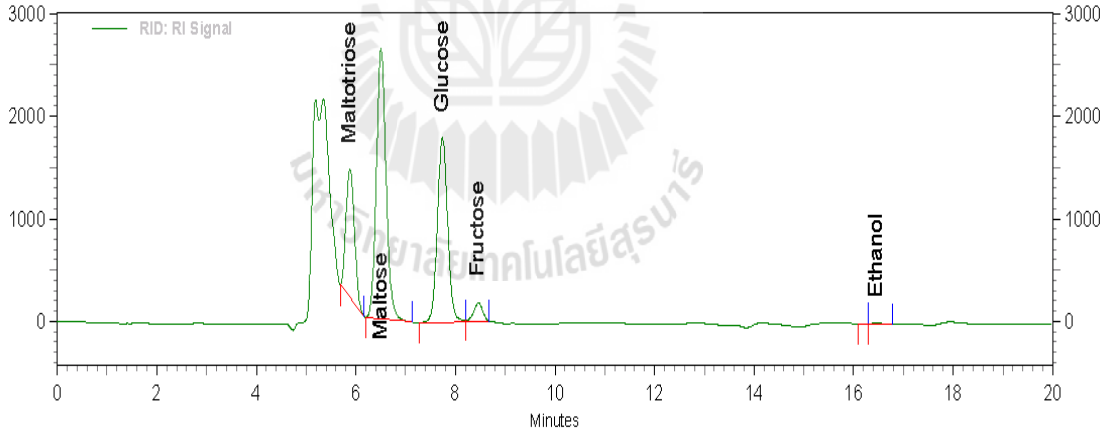


Figure 8C HPLC chromatogram of fermentable sugars in 3-50 wort at 0 h using Vertisep^{TM} OA column with DI water mobile phase and RI detector.

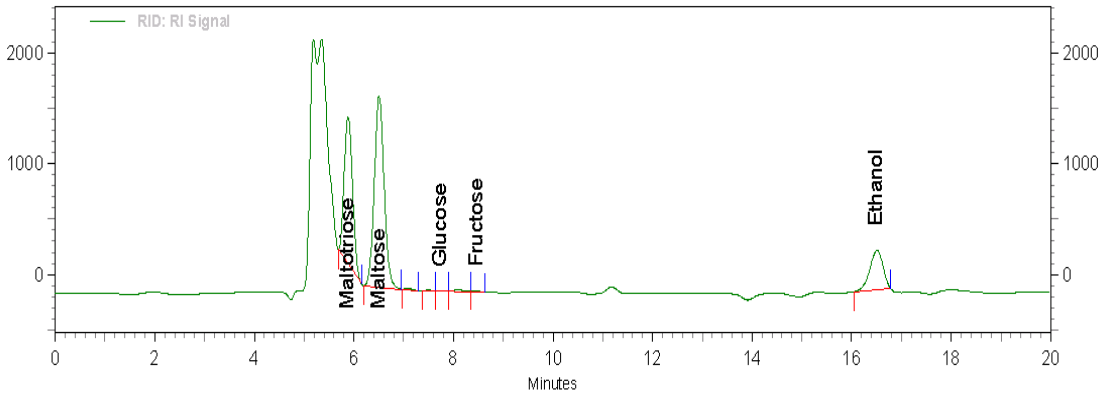


Figure 9C HPLC chromatogram of fermentable sugars in 3-50 wort at 48 h using Vertisep^{TM} OA column with DI water mobile phase and RI detector.

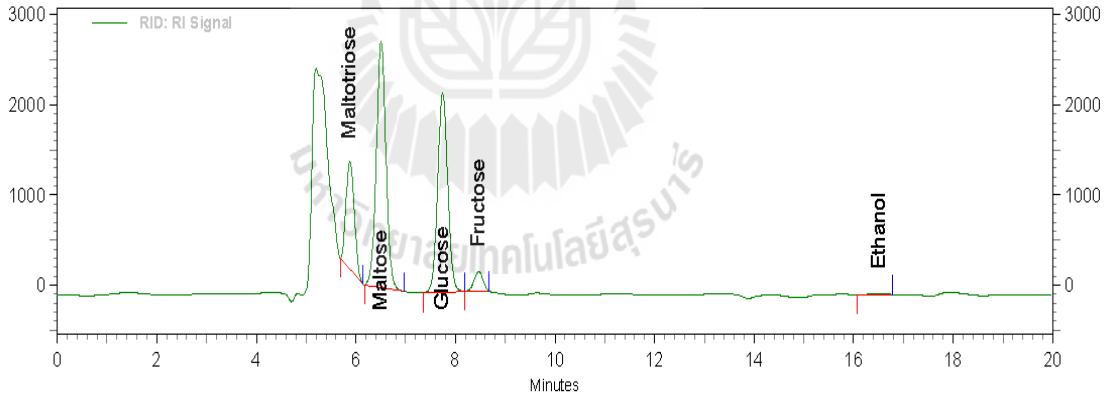


Figure 10C HPLC chromatogram of fermentable sugars in 5-50 wort at 0 h using Vertisep^{TM} OA column with DI water mobile phase and RI detector.

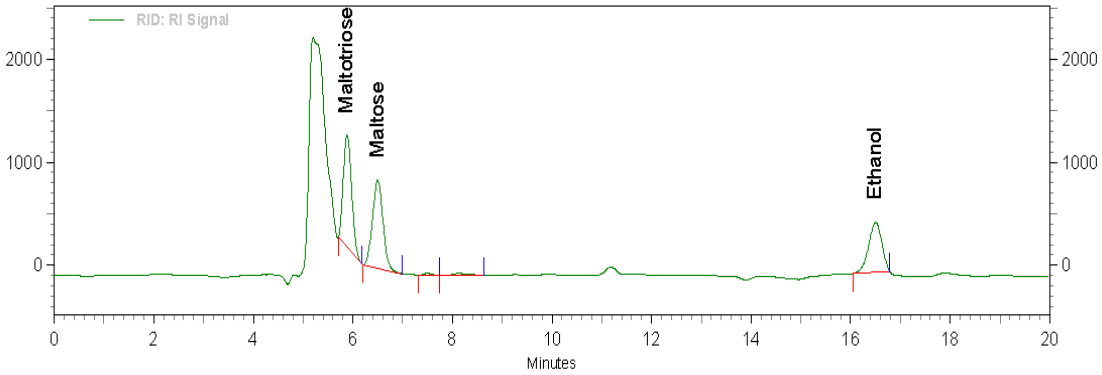


Figure 11C HPLC chromatogram of fermentable sugars in 3-50 wort at 48 h using Vertisep^{TM} OA column with DI water mobile phase and RI detector.

APPENDIX D

LIST OF PRESENTATION

Poster presentation

Kongkaew, A., Wanapu, C. and Usansa, U. (2010). **Response surface optimization of wort production for brewing from rice malt using commercial enzymes and malt barley.** The 16th Asian Agricultural Symposium and 1st International Symposium on Agricultural Technology: Sufficiency Agriculture, August 25 – 27, 2010, Faculty of Agricultural Technology, KMITL, Bangkok, Thailand.

Kongkaew, A., Wanapu, C. and Usansa, U. (2010). **Beer production from rice malt based in pilot scale brewing : chemical and sensorial properties approach**. The 3rd SUT Graduate Conference 2010, November 21 – 23, 2010, Suranaree University of Technology, Nakhonratchasima, Thailand.

P1-18

Response Surface Optimization of Wort Production for Brewing From Rice Malt Using Commercial Enzymes and Malt Barley

KONGKAEW, A.[1], C. WANAPU[1]* and U. USANSA[2]
[1]School of Biotechnology, Institute of Agricultural Technology,
Suranaree University of Technology, Nakhonratchasima 30000, Thailand
[2]Department of Biotechnology, Faculty of Agro-Industry, Kasetsart University,
Bangkok 10900, Thailand
*Corresponding author: wanapu@sut.ac.th

Abstract

The present experiment aims to increase rice malt ratio for wort production by using commercial enzymes (TermamylSC®, heat-stable α-amylase; and Neutrase®, bacterial protease) in wort production step. The experimental design was determined using response surface methodology (RSM) using a quadratic polynomial model. Small-scale mashing of 26 experiments were carried out. The temperature-program was 45°C×10 min, 50°C×60 min, 63°C×40 min and 95°C×60 min. Germination time of rice, rice malt ratio, α-amylase and protease were selected as the independent variables. From responses plot, α-amylase exhibited the essential for extraction and improved the filtration volume due to the saccharification activity. The proportion of 75% rice malt with α-amylase illustrated the maximum filtrate volume. Addition of bacterial protease increased the amount of FAN content. However, germination time of rice demonstrated the highest impact on FAN in wort as well as ratio of malted barley illustrated the increase of fermentable sugar in wort. Conditions to obtain the appropriate wort for brewing base on germination time and rice malt ratio were determined.

Keywords: rice malt, wort production, response surface methodology

มหาวิทยาลัยเทคโนโลยีสุรนารี

ARG-P-19

Beer production from rice malt based in pilot-scale brewing: chemical and sensorial properties approach

Artit Kongkaew[1], Chokchai Wanapu[1] and Ulaiwan Usansa[2]
[1]School of Biotechnology, Institute of Agricultural Technology,
Suranaree University of Technology, Nakhon Ratchasima, Thailand.
[2]Department of Biotechnology, Faculty of Agro-Industry,
Kasetsart University, 10900, Thailand.
E-Mail: artit_kk@hotmail.com

Abstract

Production of beer from rice malt at difference ratios (50, 70 and 90%) in 50 l scale brewing plant was carried out. Commercial enzymes (TermamylSC® as heat-stable α-amylase, and Neutrase® as acterial protease) as well as barley malt were added for improving the quality of rice beer. Fifth days of germinated rice at difference ratios were brewed. Maturation and carbonation step of beer were completed. The amount of ethanol in final beer was 3.18 - 4.00 % (v/v), might be due to the lack of maltotriose utilization in fermentation step. Quantity of rice malt ratios was direct variation with the color of beer at 20 EBC for 50% rice malt and 30 EBC for 70 and 90% rice malt, which defined as the dark lager beer. Rice malt beer contained isoamyl alcohol of approximately 600 ppm as well as 350 - 600 ppm octanoic acid, which are the main reason of off-flavor in beer. The overall impression score of 50% rice malt was at 3.0 defined as good, while 70% and 90% rice malt beer were at 2.0 defined as normal and drinkable.

Keywords: Pilot-scale brewing, Rice malt, Chemical property, Sensory analysis

มหาวิทยาลัยเทคโนโลยีสุรนารี

BIOGRAPHY

Name Mr. Artit Kongkaew

Date of Birth 23 April 1984

Place of Birth Nakhonratchasima, Thailand

Education

2002-2005 B.Sc. (Food Technology), Suranaree University of Technology, Nakhon Ratchasima, Thailand

Experience

2005 Research assistant of the research project entitled **"**Effects of Conjugated Linoleic Acid (CLA) in milk" at Suranaree University of Technology, Nakhon Ratchasima, Thailand

2005 Research assistant of the research project entitled "Study in fresh-water fish, which produced Conjugated Linoleic Acid (CLA)" at Suranaree University of Technology, Nakhon Ratchasima, Thailand

2006 Scientist in Quality control at Thai Airways, Catering Service, Quality Assurance Department, Bangkok, Thailand.

2007 Research assistant of the research project entitled "Possibility of using Thai herbal medicine to correct male incompetence" at Suranaree University of Technology, Nakhon Ratchasima, Thailand.

Awards

2005 Joining in "Nestle FoStat Quiz Bowl 2005 technical matter contest.