MAIZE MALT SUPPLEMENTATION OF BARLEY FOR THE NEW BEER PRODUCTION

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การผลิตเบียร์ชนิดใหม่จากบาร์เลย์เสริมด้วยมอลท์ข้าวโพด

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

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ทือ ถิ อัน เล : การผลิตเบียร์ชนิดใหม่จากบาร์เลย์เสริมด้วยมอลท์ข้าวโพด (MAIZE MALT SUPPLEMENTATION OF BARLEY FOR THE NEW BEER PRODUCTION) อาจารย์ที่ปรึกษา : รองศาสตราจารย์ ดร.โชคชัย วนภู, 122 หน้า

เบียร์เป็นเครื่องดื่มแอลกอฮอล์ที่ได้รับความนิยมเป็นอย่างมากในโลก จากความต้องการของผู้บริโภค นำมาสู่การพัฒนาให้ได้เบียร์ที่มีความแปลกใหม่ของกลิ่น รสชาติ และคุณภาพ การวิจัยฉบับนี้จึงมุ่งเป้าที่จะหาสภาวะที่เหมาะสมของกระบวนการผลิตเบียร์ที่มีการใช้ข้าวโพดเป็นวัตถุดิบหลัก โดยใช้เป็นแหล่งของแป้งและโปรตีน ผสมกับมอลท์บาร์เลย์สัดส่วนต่างๆ ในการผลิตเบียร์ โดยทำการเพิ่มปริมาณของข้าวโพดในระดับต่างๆ ในการศึกษาผลของการแช่เมล็ดข้าวโพดต่อกิจกรรมของเอนไซม์จากมอลท์ข้าวโพด พบว่า กิจกรรมของเอนไซม์ที่เกี่ยวของกับการย่อยแป้งและการย่อยโปรตีนเพิ่มขึ้นตามอุณหภูมิ และให้ค่าสูงสุดที่ 30 องศาเซลเซียส อย่างไรก็ตาม เมล็ดข้าวโพดที่ทำการแช่ที่อุณหภูมิ 35 และ 40 องศาเซลเซียส ให้ผลของการผลิตเอนไซม์ต่ำ นอกจากนี้กิจกรรมของเอนไซม์ขึ้นอยู่กับเวลาที่ใช้ในการแช่ โดยพบว่า การแช่ที่นานกว่า 24 ชั่วโมง มีผลยับยั้งกิจกรรมของเอนไซม์ เวลาที่ใช้ในการแช่ อุณหภูมิที่ใช้ในการงอก และเวลาที่ใช้ในการงอก มีผลต่อมอลท์ข้าวโพดที่ผลิตได้ พบว่าเอนไซม์อัลฟาและเบต้าอะไมเลส, เอนไซม์โปรติเอส, ค่า Free Amino Nitrogen (FAN), ค่า Kolbach index, ค่า Extract content และปริมาณของน้ำตาลรีดิวส์ให้ปริมาณที่สูงที่สุด เมื่อข้าวโพดถูกแช่ที่อุณหภูมิ 30 องศาเซลเซียสเป็นเวลา 24 ชั่วโมง และทำการงอกที่อุณหภูมิ 30 องศาเซลเซียส เป็นเวลา 5 วัน ซึ่งสภาวะที่เหมาะสมนี้ได้นำไปใช้สำหรับการผลิตมอลท์ปริมาณมากต่อไป

น้ำเวิร์ทจำนวน 11 ตัวอย่างที่ได้จากกระบวนการ mashing ถูกนำมาวิเคราะห์คุณภาพ โดยพบว่า ค่า FAN มีความเข้มข้นอยู่ในช่วง 290 ถึง 350 มิลลิกรัมต่อมิลลิลิตร ค่า extract content อยู่ที่ 14.74 องศาพาโต้ (°P) ปริมาณของน้ำตาลที่สามารถนำไปหมักในน้ำเวิร์ทที่ได้ในแต่ละตัวอย่างให้ผลไม่แตกต่างกันอย่างมีนัยสำคัญ สรุปได้ว่าสัดส่วนของมอลท์ข้าวโพด และมอลท์บาร์เลย์ทั้งหมดสามารถใช้ได้ในกระบวนการการผลิตเบียร์ได้

ในกระบวนการหมักเบียร์ตัวอย่าง 3 ชุดตัวอย่าง ได้แก่ 100% ของมอลท์ข้าวโพด, 50% ของมอลท์ข้าวโพด และ 50% ของมอลท์บาร์เลย์ และ 100 % ของมอลท์บาร์เลย์ ได้ทำการวิเคราะห์

สารประกอบที่สามารถระเหยได้ และทดสอบการชิมในขั้นตอนสุดท้ายเบียร์พบว่า เบียร์ที่ผลิตจาก 100% ของมอลท์ข้าวโพดได้รับการประเมินอยู่ในเกณฑ์ที่สามารถดื่มได้ และยังสามารถดื่มแก้วต่อไปได้ ส่วนเบียร์ที่ผลิตจาก 100% ของมอลท์บาร์เลย์ แสดงผลอยู่ในเกณฑ์ดีถึงดีมาก แสดงให้เห็นว่าสามารถนำข้าวโพดใช้เป็นวัตถุดิบในกระบวนการผลิตเบียร์ที่มีคุณภาพ

สาขาวิชาเทคโนโลยีชีวภาพ ลายมือชื่อนักศึกษา _____

ปีการศึกษา 2560 ลายมือชื่ออาจารย์ที่ปรึกษา _____

THU THI ANH LE : MAIZE MALT SUPPLEMENTATION OF BARLEY FOR THE NEW BEER PRODUCTION. THESIS ADVISOR : ASSOC. PROF. CHOKCHAI WANAPU, Ph.D, 122 PP.

MALTING/MAIZE MALT/MASHING/FERMENTATION/BEER/SENSORY EVALUATION

Beer is the most favourable alcoholic beverage in the world. From demand of customers by offering new beer aroma, flavour and quality improvement, the trend of this period additionally pushes breweries to the advancement of novel products. The aim of this research is to optimize the brewing process, which uses maize as a major starch and a protein source in order to increase the value of maize and make a new beer product. The effect of the steeping regime on enzyme activities of the maize malt was investigated. The enzyme activities of maize were examined during the steeping time and up to seven days of germination. The amylolytic and proteolytic activities of maize malt were maximized at 30°C. Nevertheless, the maize was steeped at 35 and 40°C retarded the enzyme synthesis. Furthermore, the enzyme activity also depended on the steeping time, a long steeping duration for more than 24 h inhibited the activities of the enzymes. In addition, the investigation of the steeping time, germination temperature and germination time were studied to determine the malt quality. The α- and β-amylases, protease, Free Amino Nitrogen (FAN), Kolbach index, extract content and reducing sugar were explored. The highest amounts were found when the maize was steeped at 30°C for 24 h and germinated at 30°C for five days. These optimal conditions were selected for producing maize malt in a larger scale.

Eleven worts obtained from suitable mashing processes were used to measure the quality. The FAN, extract content and fermentable sugar in wort were probably affected by the proportion of maize malt and barley malt. The FAN concentration was from 290 to 350 mg/mL. The extract content of each treatment was 14.74°P. On the other hand, the fermentable sugars were not significantly different in numerous types of wort. These results indicated that all ratios were appropriate for brewing.

Three ratios of wort consisting of 100% maize malt, 50% maize malt with 50% barley malt and 100% barley malt were chosen for beer fermentation. After that, the volatile compounds of the final beers were analyzed. Finally, the sensory evaluation was carried out. The result showed that the maize beer was judged as drinkable and preferred to the next glass when supplemented, and 100% barley beer showed good to very good properties. The results obtained from this research demonstrated the ability of maize as a raw material with acceptable quality for brewing technology.

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Student's Signature _____

Advisor's Signature _____

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LIST OF ABBREVIATIONS

AOAC	=	Association of Official Analytical Chemists
ADP	=	Adenosine diphosphate
ATP	=	Adenosine triphosphate
BU	=	Bitterness Unit
^{o}C	=	Degree Celsius
Co.Ltd	=	Limited Company
DI	=	Deionize
et al.	=	et alia (and others)
EDTA	=	Ethylenediaminetetraacetic acid
DMS	=	Dimethyl Sulfide
DNS	=	Dinitrosalicylic acid
EBC	=	European Brewery Convention
eV	=	Electron volt
FAN	=	Free amino nitrogen
FAO	=	Food and Agriculture Organization

LIST OF ABBREVIATIONS (continued)

g	=	gram
g/L	=	Gram per liter
GA	=	Gibberellic acid
GC-MS	=	Gas chromatography coupled with mass spectroscopy
h	=	Hour
HPLC	=	High performance liquid chromatography
kg	=	Kilogram
L	=	Liter
M	=	Molar
mg	=	milligram
mL	=	milliliter
mm	=	millimeter
mM	=	millimolar
min	=	minute
ND	=	no data
nm	=	nanometer
N	=	normality

LIST OF ABBREVIATIONS (continued)

^{o}P	=	degree Plato
PDMS	=	Polydimethylsiloxane
PSI	=	Pound square per inch
%	=	Percentage
RI	=	Reflexive index
RO	=	Reverse osmosis
rpm	=	Round per minute
R^2	=	R-square
s	=	second
SD	=	Standard deviation
SPME	=	Solid phase microextraction
SPSS	=	Statistical Package for the Social Sciences
U	=	Unit
µg	=	Microgram.
µm	=	Micrometer
USDA	=	United States Department of Agriculture

LIST OF ABBREVIATIONS (continued)

U.S.A.	=	United States of America
v/v	=	volume by volume
w/v	=	weight by volume
w/w	=	weight by weight

CHAPTER I

INTRODUCTION

1.1 Significance of the study

Beer has been a most popular alcoholic beverage in the world for thousands of years. Many confirmations demonstrated that brew was initially created by antiquated Egyptian no less than 7000 years back (Hornsey, 2003). Therefore, brewing is frequently portrayed as the oldest biotechnological processing. The ordinary beer sorts still overpower the market, the trend of this period additionally pushes breweries to the advancement of novel products. The inspiration is on both sides: customers normally seize the opportunity to discover novelties, while producers contend with each other for customers by offering new flavours and quality improvement.

Beer generally utilizes malted grain that provides additional wellsprings of sugar and protein into the wort. Nevertheless, barley does not grow in the tropical zone, for instance, Southeast Asia. Moreover, the cost of malted grain is rising. Therefore, by virtue of its lower cost, produced adjunct materials can be used to supplement malted grain. The competition has influenced the business to develop new sources of competitive advantages, requiring a ceaseless procedure of advancement. This has driven manufacturer to make and utilize types of progress or contraptions that will make open passages for new things organizations and business processes.

The using of gluten ingredients (barley and wheat) in brewing may cause harmful to the consumers who have celiac disease. Celiac disease is defined as an autoimmune disorder that causes the immune system to respond when the body is triggered by the ingestion the protein in certain grains (Bower, Sharrett, & Plogsted, 2006). The gluten-free diet is necessarily beneficial for the patient who is diagnosed with celiac disease. However, the abundance of gluten-free food is lower than the common product. The rising incidence of obesity, diabetes, celiac disease, other diagnosed food intolerances, and growing consumer awareness to address these conditions expanded the market to manufacture customised allergen-free products. The increasing of market value could attract the brewing industry and scientist pay more attention to gluten-free beer.

In the current study, the authors intend to develop the gluten-free beer in brewhouse conditions. In order to eliminate the gluten content in beer, several techniques have been applied. In addition, the beer is brewed from alternative cereals or pseudocereal material has been studied. Adjunct is defined as "anything that is not malt, yeast, hops or water" by the Germany purity law, or The Foods Standards Committee in the United Kingdom defines a brewing adjunct as "a any carbohydrate source other than malted barley which contributes sugars to the wort" (Bamforth, 2016; Stewart & Priest, 2006). For a long time, adjunct such as maize (corn), rice, unmalted grain, wheat starch and sorghum have been utilized by the breweries to give fermentable carbohydrates to the yeast. There are many purposes behind the utilization of adjunct in addition to, or instead of, barley malt, including better accessibility the materials for processing, sensory modification of the beer and probably the most basic one, a lower cost. Supplanting barley malt with adjunct is generally cost saving. It has been proven

that the use of a 30% of corn adjunct can give an 8% reduction in total production costs (this number may vary depending on the local prices of raw materials and other costs of production) (Poreda et al., 2014).

The most generally utilized extra materials are corn (maize, *Zea mays*) (46% of adjunct), rice (31%), grain (1%), or sugars and syrups (22%). The accompanying materials have been utilized as un-malted brewer's adjuncts: yellow corn meal, refined corn starch, rice, sorghum, grain, wheat, wheat starch, stick and beet sugar (sucrose), rye, oats, potatoes, cassava, triticale, and even pea starch (Bamforth, 2016).

There are many researches to investigate the effect of adjuncts in beer processing. Rice has been used as an adjunct in gluten-free beer or dilution extract. Rice consist of 85 - 90% of starch and its protein from 5 – 8%. Rice is the most commonly used in gluten-free beer, because its proteins are not considered coeliac toxic. But there is little data accessible concerning malting and fermenting with 100% rice. Usansa et al. (2011) optimized the malting conditions of black waxy (high amylopectin) and non-waxy rice (low amylopectin). The rice malts obtained had lower extract contents, showed poorer β-amylase activities but higher amounts of limit-dextrinase and α-amylase activities, than barley malt. Mayer et al. (2016) produced beer from 100% rice malt. However, the wort showed lower total nitrogen and FAN (Free-Amino Nitrogen) content and suboptimal wort sugar compositions.

Sorghum (*Sorghum bicolor*) and maize are two closely related species. The proximate composition and nutritional value of sorghum is similar to that of maize, its proteins are less digestible (Colin et al., 2016). Sorghum beer is mostly produced in Africa. In Africa, sorghum is a traditional raw material for the production of local top-

fermenting beers that are known by various names such as: Bantu beer in South Africa, dolo in Burkina Faso, and billi in Chad. These beers are of relatively low-alcohol concentration [approx. 2.5 (v/v)] and are produced without hops. They are slightly sour in taste and are drunk unfiltered (containing “bits”), mainly in rural regions (Bamforth, 2016). It is produced by souring (lactic acid bacteria fermentation), cooking, mashing, straining and fermenting (yeast fermentation). The souring process is carried out by inoculating a water suspension of ground malt with *Lactobacillus leichmannii* (Hager et al., 2014). Comparing with commercial beer, sorghum beer is rather viscous beverage. The taste is slightly sweetish and because the formation of lactic acid, it can be little sour. Its colour can be yellowish. The depth of the colour depends on the pH of the product.

Maize, the American Indian word for corn, means literally "that which sustains life" (FAO, 1992). It is, after wheat and rice, the essential oat grain on the planet, giving supplements to people and creatures and filling in as a fundamental crude material for the generation of starch, oil and protein, mixed refreshments, nourishment sweeteners and, all the more as of late, fuel. The green plant, made into silage, has been utilized with much achievement in the dairy and meat businesses. After collected of the grain, the dried leaves and an upper part, including the blossoms, are as yet utilized today to give generally great forage to ruminant creatures possessed by numerous little agriculturists in creating nations. The erect stalks, which in a few assortments are strong, have been utilized as durable fences and dividers.

Maize is the most widely used in brewing as an adjunct grain to lighten beer body, colour, and flavour. Maize grits produce a slightly lower extract than other unprocessed adjuncts, such as rice due to less dextrin material in the wort after mashing and

containing higher levels of protein and fat (Bamforth, 2016). The chemical composition of maize is quite similar with barley (76 – 80% carbohydrate, 9 – 12% protein and 4 – 5% oil (Kunze, 2004)) which is possible to produce beer. Its suitability for brewing as a malted grain is poor; therefore it is mainly used as an adjunct. However, Zweytik and Berghofer (2009) produced maize malt on a pilot scale to brew bottom-fermented beer using 100% maize. The authors reported that the resulting beer was clear, light yellow in colour, with good foam stability, and boasting a taste comparable to that of conventional beer (Zweytik & Berghofer, 2009).

Maize is one of five major crops in Thailand and Vietnam. In 2016, more than 5.2 million tons of maize was produced in Thailand and it was approximately 10.64 % increasing from the year 2015 and 13.04% increasing in the year 2012. In addition, Vietnam produced approximate 5.0 million tons maize from 2011 to 2017. Maize makes high ratio in national agriculture. Thus, maize might be an interesting source for the new beer product. The aim of this research is to investigate the technique for maize beer production.

1.2 Research objectives

The aim of this research is to optimize the brewing process, which used maize as a major starch and protein source in order to increase the value of maize and make a new product of beer. The main objective can be divided into sub-objectives as follow;

1.2.1 To optimize maize malting conditions,

1.2.2 To optimize the ratio of maize malt and barley malt for new larger beer,

1.2.3 To ferment and determine the quality of finished product.

1.3 Research hypothesis

Maize malts have the efficiency to be used for brewing as a source of carbohydrate and protein of beer. The optimization brewing process could produce new larger beer from maize.

1.4 Scope and limitation of the study

The proximate analyses of maize *(Zea mays L.)* were determined, including moisture content, protein, crude fibre, fat, ash, total carbohydrate and in addition gelatinization temperature and percentage of germination. Then, the optimum of malting conditions consist of steeping temperature, steeping time, germinated temperature and germination time were studied. After that, the finest malted maize was selected for investigation of mashing conditions and fermentation. Finally, chemical, physical and sensory analyses of final beer were determined.

1.5 Expected results

The obtained results from this research prompted more understandings of malting process for maize and the knowledge of the effect of mashing stage on wort quality could be valuable for application in the brewing industry. Besides, from this study, the new style lager product from maize, which is appropriate and acceptable qualities.

CHAPTER II

LITERATURE REVIEWS

2.1 Beer

2.1.1 Definition of beer

Beer is the most popular alcoholic beverage in the world. The manufacture of beer is called brewing, which involves the fermentation of starches, mainly derived from cereal grains—most commonly malted barley, although wheat, rice, maize (corn), and sorghum are widely used. Most beer is flavoured with hops, which provide bitterness, flavour and aroma; and act as a natural preservative.

2.1.2 Basic ingredients of beer

The essential ingredients of beer consist of water; fermentable carbohydrates, for example, barley malt which is capable to be saccharified (convert starch into sugar); yeast for fermentation, convert sugar into alcohol and carbon dioxide; and hops, for flavouring. A combination of starch source can be used, with a helper starch source, for instance, maize (corn), wheat, rice, oat, sorghum or sugar, regularly been named an adjunct, particularly when utilized as a lower-cost substitute for malted barley.

2.1.2.1 Water

Beer is composed mostly of water. Because it is the main ingredient by volume, to brew good beer, beer needs to start with good water. To keep it simple, the water is used for brewing should be free from chlorine or other chemicals, and should have some basic minerals.

There are several important ions to consider when evaluating brewing water. The principal ions are Calcium (Ca^{2+}), Magnesium (Mg^{2+}), Bicarbonate (HCO_3^-), Sodium (Na^+), Chloride (Cl^-) and Sulfate (SO_4^{-2}) can influence the taste of the water and beer. Na^+ ions can contribute a salty taste at a concentration of 150 to 200 mg/L (Taylor, 1981) and may be harsh and sour in excess, viz., greater than 250 mg/L (Comrie, 1967; Harrison et al., 1963; Van Gheluwe et al., 1984). However, at lower levels (up to 100 mg/L), Na^+ ions can produce a palate-sweetening effect, especially in association with Cl^- ions. Indirect effects of the ion on beer flavour and quality for yeast requirements, which requires the appropriate concentration of elemental for accelerated growth, increased biomass yield, and enhance ethanol production, or both. In addition to influencing mash pH, calcium ions can directly stimulate amylolytic and proteolytic enzyme activities during wort production. Ca^{2+} ions protect malt α-amylase activity against inhibition by heat, leading to increases in extract (Comrie, 1967; Taylor, 1981) and can lead to an increase of endopeptidase activity at lower mash temperatures (Stewart & Priest, 2006).

2.1.2.2 Barley

Barley (*Hordeum vulgare* L.) is one of the most ancient crops, and

it has played a role in the human development of agriculture, civilizations, and cultures and the sciences of agronomy, physiology, genetics, breeding, malting, and brewing. It is grown and used around the world (Ullrich, 2011). Cultivated barleys and various wild barleys and barley grasses are classified in the genus *Hordeum,* within the tribe Triticeae (syn. Hordeae, Hordeeae) (Briggs, 1978). As far as worldwide production, barley positions are the fourth major cereal crop (after wheat, rice and maize) with more than 149.25 million metric tons being produced every year around the world (USDA, 2017). Barley is the best known around the world today as a feed grain and as the premier malting and brewing grain (Ullrich, 2011). In this worldwide production, most of the barley crop is used as an animal feedstuff, however, around 15% is being utilized by the brewing and distilling industries (Morris & Bryce, 2000).

Barley is the major source for brewing malts, which constitutes the single most important raw material for beer production. The physico-chemical properties of barley and barley malt starches and the biochemistry of its synthesis and degradation are well known (Eblinger, 2009).

Malting barley is divided into two main species *H. vulgare* six-rowed barley and *H. distichon* two-rowed barley. The ear or inflorescence of barley contains six rows of grains. In six-rowed barleys, all six rows of grains develop. At two-rowed barleys, only two of the six rows of grains develop (Stewart & Priest, 2006).

The structure of barley kernel in longitudinally and transversely sections are illustrated in Figure 2.1, which contained embryo, starchy endosperm and bran layer. The embryo, which constitutes about 4% by weight of the barley grain (Finnie & Svensson, 2009) and is located at its proximal end. Two main tissues compose

the embryo, these are the axis and the scutellum. The axis comprises the root, shoot and node (Stewart & Priest, 2006), whereas the scutellum (a single cotyledon) functions to support in the absorption of nutrients from the endosperm during germination.

The endosperm consists of the pericarp, testa, aleurone and the starchy endosperm (Stewart & Priest, 2006). The starchy endosperm is the largest single structure of the whole grain which approximate 87% of the total grain dry weight (Finnie & Svensson, 2009) and is composed of thousands of cells. As the name, the starchy endosperm contains two types of starch granules. The large or A type granules, which are lenticular and range from 10 – 48 µm in diameter, and smaller, near-spherical B type granules and 1 – 10 µm (Shewry & Ullrich, 2014). The starchy endosperm also contains considerable amount of β-glucan (80% of cell wall) while the cell wall of wheat has been shown lower (20% of cell wall). Barley, therefore, differs from other grains in its β-glucan content in the starchy endosperm (Eblinger, 2009). This, thus, has a particular impact on malting quality. In 2013, the study of the influence of β-glucan on wort and beer viscosities was investigated. This study was aimed at the production of wort from malt at a different degree of cytolytic modification and estimation of the effect of exogenous β-glucanases addition during wort fermentation. Two types of malt, the poorly- and well-modified which β-glucans content was respectively 1000 $mg.L^{-1}$ and 384 $mg.L^{-1}$ were used to explore the impact of β-glucans in beer quality. Furthermore, the addition of enzymatic preparation with β-glucanases activity during the fermentation of wort, obtained from poorly modified malt, resulting in decreasing of beer viscosity by 5–40% (depending on exogenous enzyme concentration: 1 and 2 BGU/L Finizym® 200L, a fungal β-glucanase) and decreasing of β-glucan content by more than 90 % (Jonkova & Surleva, 2013).

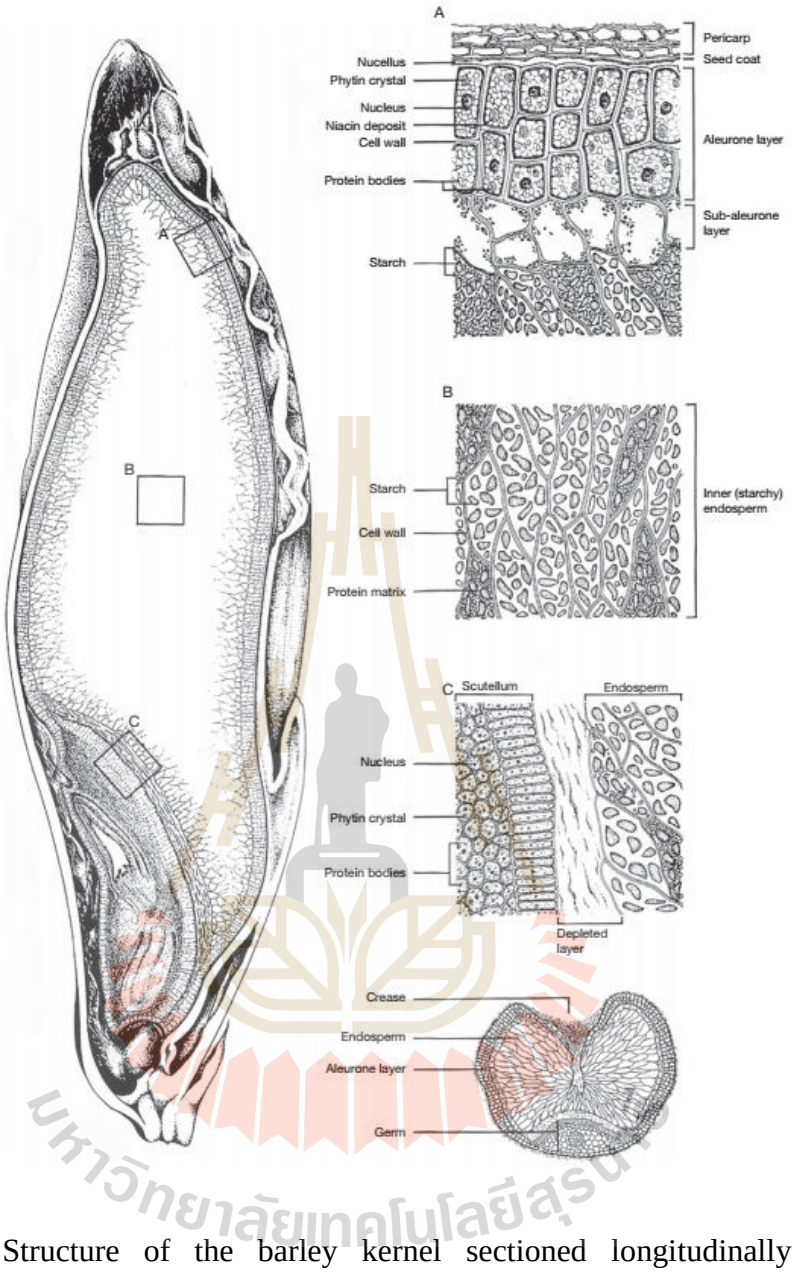

Figure 2.1 Structure of the barley kernel sectioned longitudinally (left) and transversely (right), with detailed bran layer (A), starchy endosperm (B), and embryo (C).

Source: Izydorczyk and Dexter (2004).

The endosperm is surrounded by aleurone layer which contributes about 9% of total grain dry weight (Finnie & Svensson, 2009), the most important starting point for enzyme production during malting. During germination, gibberellic

acid from germinated embryos can induce aleurone cells to produce endosperm degrading enzymes, such as a-amylase, endo-β-1,3:1,4-glucanases, limit dextrinases, endoproteases, and xylanases (pentosanases) (Stewart & Priest, 2006).

The outermost is “husk” that mostly comprise of cellulose which can act adversely on the qualities of beer including polyphenol and bitter substances.

2.1.2.3 Hops

The flavour of beer is the sole major commercial use of hops. The flower of the hop vine is used as a flavouring and preservative agent in nearly all beer made today. The flowers themselves are frequently called "hops".

Hops contain several characteristics that brewers want in the beer. Hops contribute a bitterness that adjusts the sweetness of the malt; the bitterness of beers is measured on the International Bitterness Units (IBU) scale. Hops contribute citrus, floral and herbal aromas and flavours to beer. Hops have an antibiotic impact that favors the activity of brewer's yeast over less desirable microorganisms and aids in "head retention", the length of time that a foamy head created by carbonation will last. The acidity of hops is a preservative.

The most evident contributions of hops to beer flavours is that of the so-called soft resins, chiefly the alpha acids (also known as humulones), which are ultimately responsible for the characteristic bitter taste. There are three main homologs of alpha acid humulone, cohumulone, and adhumulonedhe ratios of each varying from variety to variety. The alpha acids themselves are nonbitter, and it is only when

converted to their isomerized forms (iso-alpha acids, Figure 2.2) during the boiling process that they become bitter to taste. There are differences in the solubility of each of these iso-alpha-acid homologs with the isocohumulone being the most soluble and chemically active (Bamforth, 2016).

The other significant part of the soft resins is the β-acid fraction (Figure 2.2). The amount of β-acids is normally lower than α-acids and the ratio of α- to β-acids is variety dependent. Beta-acids are also known as lupulones and, similarly to the α-acids, exist as three homologs (lupulone, colupulone, and adlupulone). They are largely insoluble and are normally lost during boiling (leaving a maximum of 1 ppm in beer). Isomerization of β-acids is very difficult even under extreme boiling conditions; however, a small amount of the β-acids is oxidized to humulones in the hops or hop product and, being more soluble, are more likely to end up in the beer (Bamforth, 2016; Eblinger, 2009; Stewart & Priest, 2006).

The bitter resins give the beer the fine pleasant hop bitterness, aid the salubriousness, stabilize the foam and increase the biological shelf - life with their antibacterial properties (Van Cleemput et al., 2009; Zanoli et al., 2007). Maietti et al. (2017) determined antioxidant activity of flavonoids present in young hops shoot. The results showed that hop shoots represent a new source of flavonoids; therefore, they can be useful for a possible incorporation in the diet as a functional food or applied in the nutraceutical ambit. Several authors demonstrated the antibacterial and antioxidant of some biological compounds present in hops (Abram et al., 2015; Dušek et al., 2016; Leite et al., 2013; Masek et al., 2014; Natarajan et al., 2008).

The presence of the oil- and resin-rich lupulin glands, the overall

composition of fresh, dried hop cones shows them to be unlike that of other plant material, though the leafy nature of the hop petals ensures the presence of such ubiquitous substances as proteins and carbohydrates. The resinous fraction of fresh hops contains mostly the α-acids and β-acids (Figure 2.2), each of which consists of analogous series of closely related homologs.

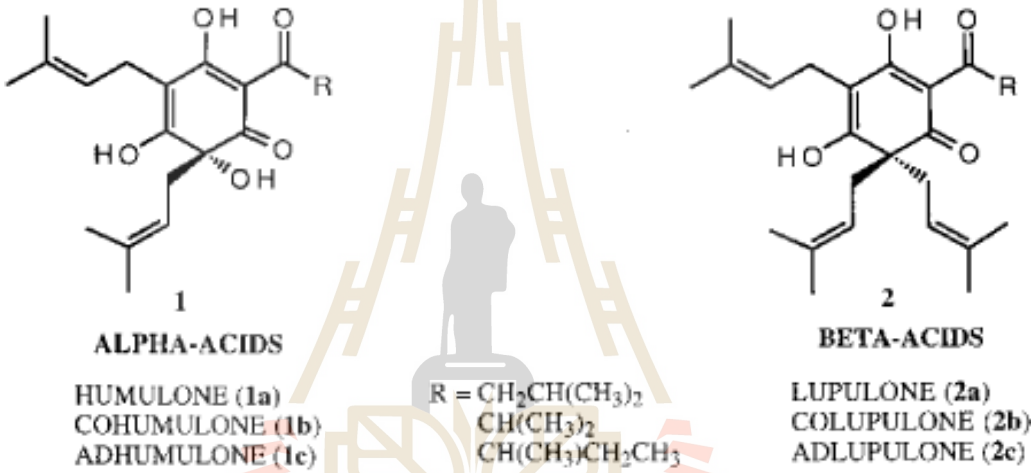

Figure 2.2 Structures of hop alpha-acids and beta-acids.

Source: De Keukeleire, 2000

2.1.2.4 Yeast

Yeast is the microorganism that is in charge of fermentation in brew. Yeast utilizes the sugars extricated from grains, which produces alcohol and carbon dioxide, and in this way transforms wort into beer. In addition to fermenting beer, yeast impacts the character and flavour. The predominant sorts of yeast used to make brew are the top-fermenting *Saccharomyces cerevisiae*, which would commonly be

utilized to produce ales, and bottom-fermenting *S. pastorianus,* which normally be utilized to create lagers. *Brettanomyces* ferments lambics, and *Torulaspora delbrueckii* ferments Bavarian weissbier. Before the role of yeast in fermentation was understood, fermentation involved wild or airborne yeasts. A few styles such as lambics rely on this method today, but the most modern fermentation adds pure yeast cultures (Baxter & Hughes, 2001; Eblinger, 2009; Stewart & Priest, 2006).

2.1.2.5 Adjunct

For a long time, adjunct, wheat, maize, rice, unmalted barley and sorghum have been utilized by the brewing industry to contribute fermentable sugar for the yeast. There are numerous explanations behind the application of adjunct in addition to, or instead of, barley malt, including better accessibility on the local market, sensory modification of the beer and probably the most critical one, a lower price. Replacing barley malt with adjuncts is usually a cost saving. In general, corn tends to give a fuller flavour to beers than wheat, which imparts a certain dryness. Barley will give a stronger harsher flavour. Both wheat and barley adjuncts can considerably improve head retention (foam). Rice will also give a very characteristic flavour to beer (Bamforth, 2006).

2.1.3 Classification of beer

There are two main categories of beer are ale and lager. This classification is based on the yeast used in the beer processing.

2.1.3.1 Ale

Ale is a one type of beer. However, hundreds of years ago the term *Ale* mentioned to a sort of drink brewed from malted barley, which was fundamentally the same as beer but without hops. The differences between Ale and other beers are yeast strain, fermentation temperature and taste. Ale is generally fermented in temperature from 15 to 24^{o}C, which is higher than lager beer. Ale is produced with top-fermented yeast, mostly *S. cerevisiae*. The term "top-fermenting" describes the flocculation tendency of the yeast. An ale yeast at ideal temperature will tend to bunch together and rise to the surface. The taste of Ale is very particular. Generally speaking, the ale has more ester compounds and other secondary flavour and aroma products. These compounds make ale fruity. Ale is a big family with numerous similar but different members, including some famous types of ale namely brown ale, pale ale, and India pale ale (Pandey et al., 2017).

Pale Ale – Whether American or English, the "pale" was clipped on long ago to distinguish it from the dark colour of Porters. American and English styles differ, but generally, they are gold or copper coloured and dry with crisp hop flavour.

India Pale Ale (IPA) – Pale ale with intense hop flavour and aroma and slightly higher alcohol content.

Brown Ale – These distinctively northern English style ales have a strong, malty center and can be nutty, sweet and very lightly hopped. They are medium bodied and the name matches the colour of the ale.

Stout (Guinness and Murphy's are dry Irish stouts) – Thick,

black opaque and rich. Stouts draw their flavour and colour from roasted barley. They often taste of malt and caramel, with little to no hop aroma or flavour.

Porter – Very similar to stout but made from, or largely from, unroasted barley. Sweet and dark brown in colour with hints of chocolate and a sometimes-sharp bitterness.

Wheat Beer – Germans take their beer very seriously, so much that it is required by law to use top-fermenting yeast in wheat beer. It must be made from at least 50% wheat malt. Wheat proteins contribute to a hazy, or cloudy appearance and are commonly unfiltered, leaving yeast sediment in the bottle. They are light coloured, full flavoured and the unique yeast strains produce flavours like banana, clove and vanilla.

Hefeweisen – The most commercially successful type of wheat beer. In the US they are regularly served with a lemon wedge to cut the intense yeast flavour.

2.1.3.2 Lager

Lager is a favourable beer type. The term “lager” means storage (from the German *largern*), which indicates that lager beer is generally stored at low temperature for quite a long time after the fermentation. Not only that, the fermentation temperature is also lower than ale beer (7 – 13^{o}C). Another huge contrast among ale and lager is the yeast that is utilized. *S. pastorianus* - so called “bottom fermenting” or “lager” yeast - is more fragile. It ferments throughout the body of the beer wort and

settles to the bottom of the vessel at the end of that process. The wonder of bottom-fermenting yeast is that, in addition to being very fragile, it attenuates more slowly and to a lesser extent than ale yeast. Because the yeasts and conditions used in lager beer fermentation are very different from those of ale beer, the taste of lager is therefore different too. This results in better clarification, a more full-bodied beer with far fewer esters and a better and more mellow palate. The final result of all that is the wonder of so-called "lager" beer, a beer that is crisper in character and less fruity in aroma than ale (Pandey et al., 2017).

Amber/Red Lager (Yuengling, Killian's, Brooklyn Lager) – More malt and darker than their lighter lager relatives, usually amber to copper coloured. Flavour profiles vary considerably between breweries. Nine times out of ten when a beer label says no more than "Lager" it is an amber.

Pilsner (Beck's, Labatt Blue, Warsteiner, Pilsner Urquell) – Conceived in Czechoslovakia, easily the world's most popular beer style. Pilsners are pale, straw coloured and crisp with medium body and more hops than traditional lager, but typically smooth and clean.

Bock (Sam Adams Winter Lager) – Of German origin, brewed in the fall to be enjoyed in the winter or spring. A stronger lager with heavy malt, medium to full bodied, lightly hopped and dark amber to brown in colour.

Doppelbock – or "double" bock is stronger and darker than bock, sweeter with more malt and a little higher in alcohol content.

Oktoberfest – indicates the Vienna style of "Marzen" beer, the German word for "March". These are brewed in the spring and stored to serve in autumn.

They have a toasted quality with a sweet tinge, robust malt flavours, and a deep amber hue.

2.2 Maize

Maize is the most important cereal grain in the world, providing nutrients for humans and animals and serving as a basic raw material for the production of starch, oil and protein, alcoholic beverages, food sweeteners and, more recently, fuel (FAO, 1992).

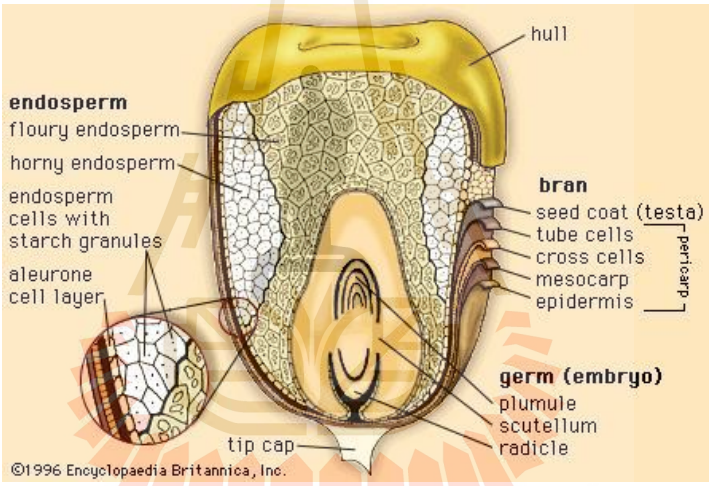

Figure 2.3 Maize kernel structure: longitudinal section enlarged approximately 30 times.

Source: FAO, 1992

Maize (*Zea mays* L. spp *mays*) belongs to the grass family (*Gramineae*) and is a annual plant growing up to 4 m tall. The female inflorescences, the ears, develop in the leaf axis on the stalk, which terminates in the male inflorescences, the tassel (Australia Goverment, 2008). The maize kernel is known botanically as a caryopsis; a single grain contains the seed coat and the seed, as shown in Figure 2.3. The figure also shows the

four major physical structures of the kernel: the pericarp, hull or bran; the germ or embryo; the endosperm; and the tip cap (dead tissue found where the kernel joins the cob). The gross anatomy and the microscopic structure of these anatomical components were well described by Wolf et al. (1969). They also studied the structure of the improved opaque-2 maize - a regulates the expression of many members of the zein multigene family of storage proteins and found differences between its endosperm and that of common maize. The protein matrix was thinner and there were fewer and smaller protein bodies, since there is a restriction in zein synthesis in opaque-2 maize. Robutti et al. (1974) reported on the protein distribution, amino acid content and endosperm structure of opaque-2 maize.

Table 2.1 Proximate chemical composition of main parts of maize kernels (%).

Chemical component	**Pericarp**	**Endosperm**	**Germ**
Protein	3.7	8.0	18.4
Ether extract	1.0	0.8	33.2
Crude fiber	86.7	2.7	8.8
Ash	0.8	0.3	10.5
Starch	7.3	87.6	8.3
Sugar	0.34	0.62	10.8

Source: Watson, 1987

Maize is harvested with a water content of 25 to 30% and brought to a water content of 10 to 14% by drying. The dry matter of maize consists of 76 – 80% carbohydrate, 9 – 12% protein, 4 – 5% oil and small amounts of crude fibre and inorganic substances (Bamforth, 2006; Eblinger, 2009; Kunze, 2004; Stewart & Priest, 2006).

According to the chemical compositions in the kernel, carbohydrate and protein are main components of the grain and play an important role to maize properties and to the brewing process. The major chemical component of the maize kernel is starch. The starch in maize is made up of two glucose polymers: amylose, an essentially linear molecule, and amylopectin, a branched form. The composition of maize starch is genetically controlled. In common maize, with either the dent or flint type of endosperm, amylose makes up 25 to 30% of the starch and amylopectin makes up 70 to 75% (FAO, 1992). Waxy maize contains a starch that is 100% amylopectin. Gelatinization takes place during mashing. Pre-gelatinized Maize flakes (as well as raw grains of rice and barley) are produced through steaming, rolling and drying. Apart from its competitive price (O'Rourke, 1999), the application of maize flakes might shorten the mashing time significantly. Maize flakes can be added to throw in similar fractions as the other starchy grain, rice. A comparison of raw grains of maize, sorghum and barley showed no differences with respect to wort properties if the ratio of raw cereal did not exceed 5%. If higher ratios are applied, protein wort concentration decreases proportional; slightly less in the case of maize, however (Agu, 2002). In principle, free amino nitrogen (FAN) should not drop under 150 mg/L (referring to 12 wt%) in raw grain worts, to ensure proper fermentation. If higher ratios of unmalted grains are to be applied, additional nitrogen sources must be added. Ten percent malt addition is sufficient to liquefy maize starch at 78°C. At higher temperatures near the boiling temperature, the hydrolytic enzymes are inactivated quickly and a retrogradation of starch takes place after gelatinization, resulting in incomplete liquefaction. Subsequently, starch is less accessible to enzymatic degradation. It is noteworthy that the addition of maize derivatives has a significant impact on the sensory properties of the beer (Eblinger, 2009).

2.3 Beer production

There are four main stages for beer processing: malt production, wort production, fermentation and beer ageing.

2.3.1 Malt production

Malt production is the first step in beer production. The purpose of malting is to produce enzymes in the germinating barley kernel and causes certain changes in its chemical constituents, which is referred to as solution or dissolving (Kunze, 2004). 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 steps of malting are usually given as steeping, germination and kilning.

2.3.1.1 Steeping

Steeping has two major functions: raise the moisture content from ±12% in different steps to ±40% to initiate germination and wash the grain and remove germination inhibitors as well as all floating material by skimming. To initiate germination as quickly as possible the barley must be adequately supplied with both water and oxygen during steeping. The rate of water uptake depends on time, temperature and physiology of grain, for example, size, thickness and variety.

Wijngaard et al. (2005) reported that the steeping time affected to the final buckwheat malt qualities, an increased steeping time increased malting losses,

total β-amylase activity and Kolbach index; on the contrary, total nitrogen, friability and viscosity of consequent congress wort were decreased.

Common steeping temperatures are about 12 – 18°C. Below these temperatures, the grain will be germinated at a lower rate and the insufficient of oxygen can occur at a higher temperature. Montanuci et al. (2016) mentioned that effect of steeping time and temperature on malting process, level of β-glucan decreased with increasing temperature and hydration time and the amount of α- and β-amylase increased. The solubility rate increased the hydration time and temperature.

As the grain hydrates, it swells to 1.4 times its original volume. By blowing air into the base of the steeping vessel it is possible to prevent packing and the barley is mixed. This also adds oxygen. The oxygen is needed by the kernels for respiration. A lack of oxygen may provoke CO_2 accumulation followed by fermentation and therefore a poisoning of the germ. Infestations of microbes are undesirable. They compete with the grain for oxygen and reduce the percentage germination. Some microorganisms like *Fusarium* spp. produce mycotoxins (Hofer et al., 2016), which are regulated by limit values for human nutrition and thought to cause gushing. It is possible to use agents like mineral acids, potassium and sodium hydroxides, potassium permanganate, sodium metabisulfite or formaldehyde, but they are subject to local regulations and add costs to the process (Eblinger, 2009).

2.3.1.2. Germination

Germination is a process used to produce malt for the brewing. The germination is started 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 (Colin et al., 2016). The time course of major events associated with seed germination and seedling growth is showed in Figure 2.4. The uptake of water by the dry, quiescent seed is shown in three phases: rapid at first (phase I) and then a plateau (phase II), followed by a second rise coincident with the beginning of seedling growth (phase III). The time for events to be completed varies from hours to many weeks, depending on the plant species and the germination conditions.

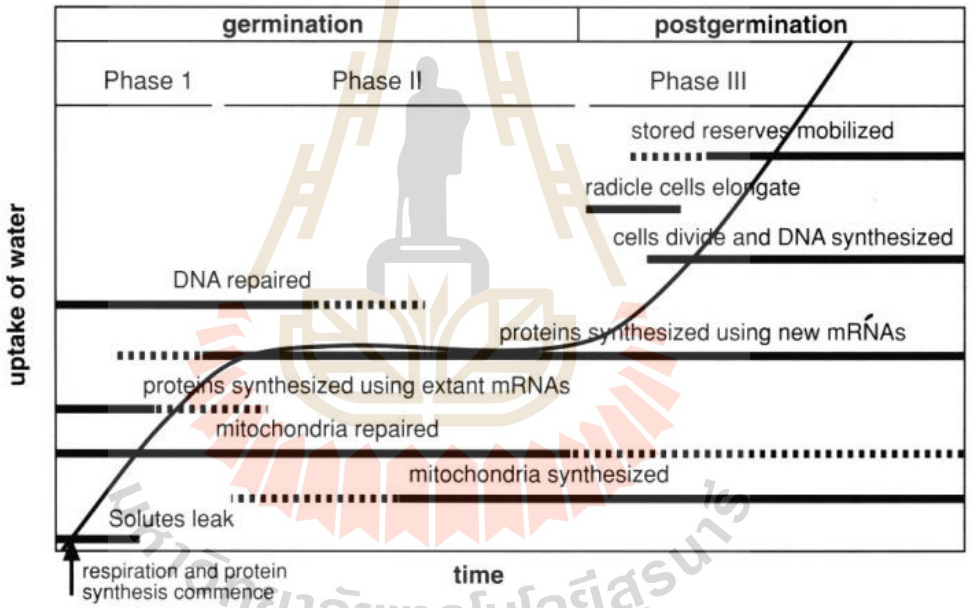

Figure 2.4 Time course of major events associated with seed germination and seedling growth.

Source: Srivastava, 2002

The step of modification of barley grain is illustrated in Figure 2.5. The steeped grains are transferred to the germination box or vessel. The objectives of germination are: controlled breakdown of cell walls and matrix proteins, produce

optimal level of hydrolytic enzymes, hydrolyze certain barley reserves, minimize loss of potential extract from growth and respiration while achieving optimal modification, produce balanced, well - modified green malt for kilning (Eblinger, 2009; Hui et al., 2007; Kunze, 2004).

The barley kernel consists of three main regions: the germ, the endosperm and the covering. Modification starts at the scutellum. The scutellum and the epithelium are thin layers between the germ and the endosperm. The enzyme induction is caused by gibberellin hormones. Gibberellic acid (GA) induces the production of different hydrolysing enzymes in the aleurone layer which covers the whole endosperm. The products of endosperm breakdown (sugars, amino acids, etc.) together with materials from the aleuronic layer (phosphate, metal ions, etc.), are needed for the growing germ.

There are many factors that can affect the germination stage such as time, temperature, moisture and variety. Morrall et al. (1986) studied the effect of germination conditions on sorghum malt quality. Germination time, temperature, moisture and the three possible pairwise interactions all had a highly significant effect on malt diastatic power (DP: the amount of beta-amylase activity), free α-amino nitrogen and extract. Malting loss was highly significantly affected by germination time and moisture and their pair-wise interaction. However, over the range examined, germination temperature had no significant effect on malting loss. In general diastatic power, free α-amino nitrogen, extract, and malting loss all increased with germination time.

Different grains have different malting conditions, which related to the genetic background of each cereal; for instance, barley is well known the ability

to grow at 17-18ºC (Kunze, 2004).

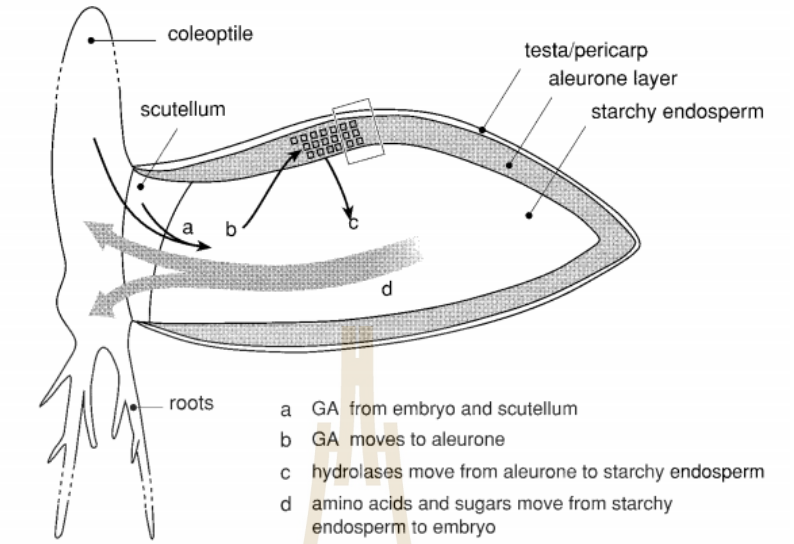

Figure 2.5 A sagittal section of a germinated grain showing the movement of GA from the embryo to the aleurone layer where the hydrolyzing enzymes are activated.

Source: Srivastava, 2002

2.3.1.3 Kilning

The green malt is transferred to the kiln. Kilns have a false bottom where large quantities of hot air can pass through to dry the green malt. There are different types of kilns. Common modern kilns are high- performance kilns with heat recovery systems and different numbers of floors. the objectives of kilning are: terminate the modification process and the growth of the plant, reduce moisture to levels suitable for grain storage, conserve enzyme complexes developed during malting, develop colour and flavour (both taste and aroma) characteristics as required by the brewer. The process

can be managed by the temperature of fresh air and performance of the fan. By using lower kilning temperature, the level of diastase and cellulose activities were a significant increase in malted sorghum (Agu et al., 1996). Phiarais et al. (2005) investigated the effect of kilning on α-amylase, β-amylase (total and soluble), β-glucanase and protease activities in buckwheat malt. All enzymatic activities were found to decrease during the kilning stage. Results indicated that after prolonged kilning at 40°C, inactivation of hydrolytic enzymes occurred; two-stage kilning for shorter periods is recommended. There are several types of malt which classified by kilning temperature, for example, light malt, dark malt or roasted malt, caramel malt, etc. The light malt is dried under low temperature which not higher than 80-85°C. It has a good odour and provides good stability beer. Dark malt is firstly dried at low temperature to remove water and increase temperature to be 100-110°C for dark colour. Colour development results from the reaction between sugar and amino acids at high temperature to form melanoidins by Maillard reaction. 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.

In broad terms, reducing sugars interact with amino compounds, (amino acids, simple peptides) or proline to yield initially Schiff's bases, but in later stages, aldosamines and ketosamines are formed via Amadori rearrangements. The latter products may condense with another sugar to yield diketosamines (Figure 2.6). Diketosamines are unstable and break down to give a range of products including hydroxymethylfurfural and reductones. Some of these products interact and polymerize to yield melanoidins and a range of small-molecular-weight substances. Other reactions break down amino acids via the Strecker degradation route. Many of the lower-

molecular-weight substances have important flavour and aroma characteristics, while the high-molecular-weight melanoidins are coloured. Melanoidins from darker malts have higher molecular weights than those from pale malts. Reductones will consume oxygen and so stabilize beer. Thus high-temperature reactions produce high-molecular-weight substances that are coloured, such as melanoidins, and low-molecular-weight substances that contribute to aroma and flavour. This last group includes acids, alcohols, aldehydes, ketones, esters and O-, S- and N-containing heterocyclic substances (Briggs, 1998).

2.3.2 Wort production

The most important process in beer production is the fermentation of the sugars contained in the wort to form alcohol and carbon dioxide. To provide the necessary conditions for this stage, the initially insoluble components in the malt must be converted into soluble products, and in particular soluble fermentable sugars must be produced. The formation and dissolving of these compounds are the purposes of wort production. It provides the starting point for fermentation of the wort in the fermentation and storage cellars.

2.3.2.1 Malt milling

In order to give the malt enzymes the opportunity, during mashing, to act on the malt contents and break them down, the malt must be broken into small fragments. This process is called milling. Milling is a mechanical process and during it the

husks must be treated carefully because they are used as a filter material during lautering.

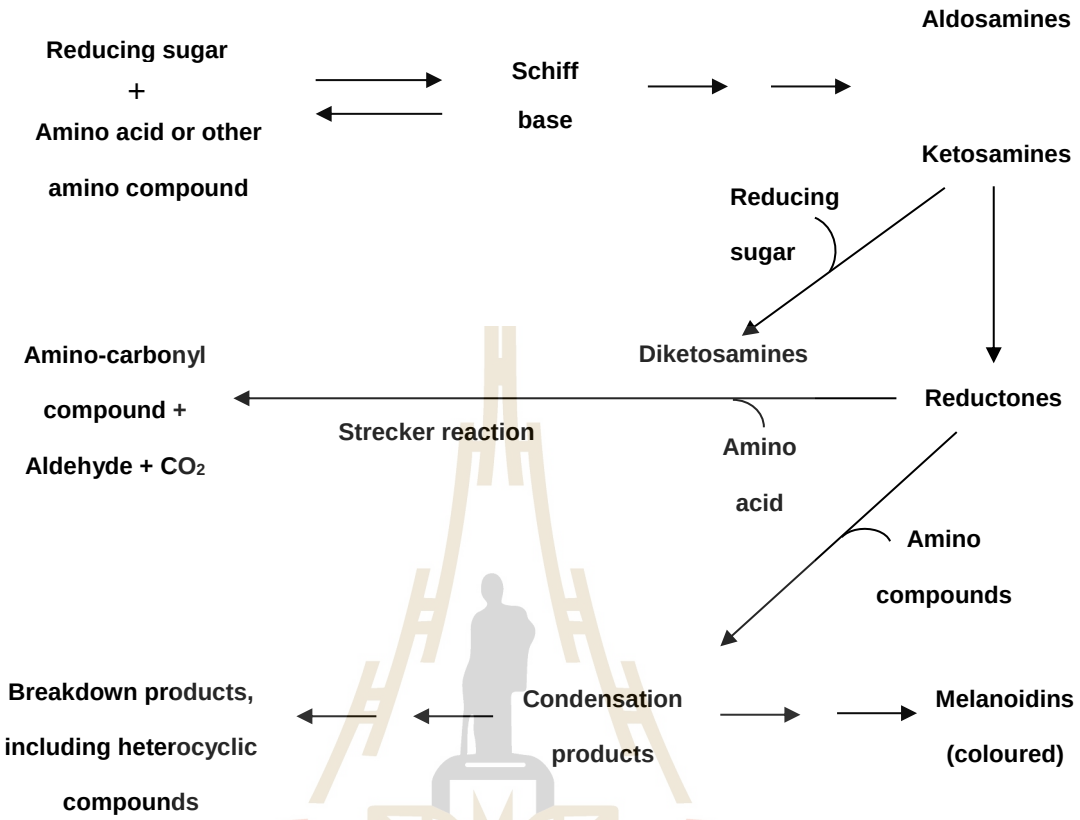

Figure 2.6 Some of the chemical steps involved in the formation of the melanoidins and some flavour and aroma substances.

Source: Briggs, 1998

2.3.2.2 Mashing

Mashing is the most important process in the wort production. During mashing the grist and water are mixed together (mashed) and the content of the malt are thereby brought into solutions and with the help of enzymes, the extract is obtained. The purpose of mashing is to completely degrade the starch to sugar and

soluble dextrins. As a result of this other extract substances are also produced. Most of the extract is produced during mashing by the action of enzymes which are then allowed to act at their optimum temperatures.

During mashing the amylolytic enzymes in the malt break down the starch into fermentable sugars. In this step, α-amylase and β-amylase play a major role in starch hydrolysis. Alpha-amylase has an optimal temperature in the range of 70 - 75^{o}C and β-amylase is around 55-60^{o}C. The protease enzyme can work at temperature 45 - 50^{o}C (Eblinger, 2009; Hui et al., 2007; Kunze, 2004; Stewart & Priest, 2006).

Mashing methods can be classified into two types of processes: infusion and decoction processes. In infusion processes, the entire mash is heated up (with appropriate rest periods) to the final mashing temperature. In decoction processes, the temperature is increased by moving part of the mash from the mash converter to the mash cooker where it is boiled (possibly also with carbohydrate rest periods in the mash cooker).

Muller (1991) studied the effect of mashing temperature and mash thickness on wort carbohydrate composition, mash performance and enzyme activity. When increasing the mashing temperature from 65^{o}C to 80^{o}C had only a slight effect on extract but reduced wort fermentability from over 70% to less than 30%. At 85^{o}C and over, when the temperature had a significant effect on α-mylase, as well as on beta-amylase, the extract was lost and starch was present in the wort. Thin mashes contained more starch and fewer fermentable sugars than did thick mashes at the same temperature. Igyor et al. (2001) investigated the impact of mashing methods on sorghum wort composition and beer flavour. The infusion mashing was carried out at different

temperature; 65, 80 and 100°C. Wort composition and development of flavour compounds were higher in sorghum malt mashed with either decantation at 80 or 100°C than infusion at 65°C.

The mashing operation will influence the alcohol content of the beer, the concentration of unfermented sugars in the beer, the peptide and amino acid profiles of the wort, the yeast nutrient concentration. In addition, the buffering capacity and pH of the wort and beer, the β-glucan content of the beer, and some beer physical properties such as foam, colour, and clarity were affected (Rehberger & Luther, 1995).

2.3.2.3 Lautering

At the end of the mashing process, the mash consists of a watery mixture of dissolved and undissolved substances. The aqueous solution of the extract is called wort, the insoluble part is referred to as the spent grains. The spent grains consist essentially of the husks, the seedling and other materials which do not go into solution on mashing or have again been precipitated during wort boiling. Only the wort is used for beer production and for this purpose it must be separated as completely as possible from the spent grain. This separation process is called lautering.

2.3.2.4 Boiling and hopping

In the kettle, hops or hop extracts are added and the wort is boiled quite vigorously. This has numerous effects:

i) Wort sterilization and enzyme inactivation

Only a short boiling time is necessary to obtain a sterile solution. The microflora of the malt, hop, and other adjuncts are readily destroyed. The inactivation of residual enzymes, which survived the mashing process, is also necessary to fix the wort composition.

ii) Extraction and isomerization of hop components

Bitter hops (hop cones, hop pellets type 90 or 45) are added at the start of the boiling process. It is necessary to sustain a high temperature over a certain time to obtain a high isomerization yield of the α-acids.

iii) Hot break formation

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 to the destruction of disulfite bridges, which convert to free thiol-groups. These can be reacted with thiol-groups of another proteins and peptides.

iv) The Maillard reaction

During wort boiling, the Maillard or nonenzymatic browning reaction is rather intensive, resulting in the production of various volatile and nonvolatile aroma compounds and coloured melanoidins.

v) Formation of colouring substances

Wort boiling results in an increase in wort colour: typically 4 EBC units for a light-coloured beer. This increase is due to the formation of melanoidins, the caramelization of sugars and the oxidation of polyphenols.

vi) Removal of unwanted volatiles

During malting, S-methylmethionine (SMM) is formed. This compound is the precursor of dimethylsulfide (DMS), which gives an unpleasant smell and taste when present in the finished beer.

vii) Acidification of the wort

Upon boiling, the wort becomes slightly acidic (typically 0.1–0.3 pH units for a classical boiling process) due to the formation of melanoidins, the addition of hop acids, the precipitation of alkaline phosphates and the acidification action of Ca^{2+} and Mg^{2+} ions with phosphates.

2.3.3 Fermentation

Fermentation means to metabolize substrates into products by the activity of microorganisms and simultaneously to gain energy. In this stage, yeast transfers sugars to ethanol and CO_2. During this process, the formation of fermentation by-products, which have a considerable effect on the aroma profile and the taste of the resulting beer. Fermentation is started by adding yeast to the wort– a process called pitching. To transform wort into beer, the sugar in wort must be fermented by yeast to ethanol and carbon dioxide. This also results in the formation of the fermentation by-products which have a considerable effect on the taste, aroma and other characteristic properties of the beer.

$$C_6H_{12}O_6 \rightarrow 2CO_2 + 2C_2H_5OH$$

Brewery yeasts are mainly two types, called top - fermenting (*S. cerevisiae*)

and bottom - fermenting yeast (*S. uvarum* var. *carlsbergensis* and *S. bayanus*). The *Saccharomyces* yeasts are facultative anaerobes, which means that they can easily adjust their metabolism from aerobic to anaerobic conditions.

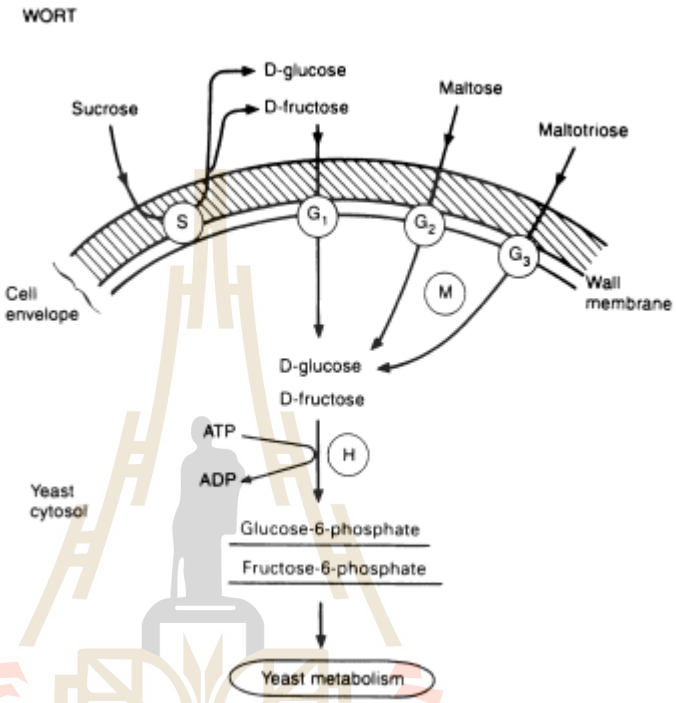

Figure 2.7 The uptake of wort fermentable sugars by yeast.

Source: Lewis and Young (2002)

During fermentation, yeast utilizes the fermentable sugars in brewer's wort are glucose, fructose, sucrose, maltose and maltotriose. The uptake of fermentable sugar by yeast is illustrated in Figure 2.7. Glucose and fructose are transported into the cell. Sucrose is hydrolyzed outside the cell to its constituent glucose and fructose. The enzyme invertase, responsible for the hydrolysis of sucrose, is located in the outer layers of the cell wall. The maltotriose and disaccharide maltose are transported into the cell and hydrolysed to glucose (Lewis & Young, 2002). The cell envelope is the wall (hatched area) and membrane (single line). G1, glucose permease (in reality, there are

at least 2 types of glucose transporters, one operating when glucose levels are high and the other at low glucose); G2, maltose permease; G3, putative maltotriose permease; S, invertase (sucrase) covalently attached within the cell wall; M, intracellular maltase (a glucosidase); H, hexokinase; ATP, adenosine triphosphate; ADP, adenosine diphosphate.

Yeast doubles or triples its mass during fermentation. For the build-up of cell substances yeast needs mostly amino acids, which it either takes from the fermenting substrate or must synthesize by itself. Apart from proteins, lipids have to be synthesized for yeast propagation because they are important components of the cell wall and are also needed for the uptake of nutrients. Molecular oxygen is necessary for the synthesis of these lipids from acetyl coenzyme A. Wort itself contains only few lipids. Finally, yeast also requires minerals for the stabilization of its enzyme systems (Eblinger, 2009).

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 flavour and stability.

Yeast has an effect on the flavours and aroma of the beer. The flavours 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 (Preedy & Watson, 2004).

2.3.4 Ageing and finishing

Ageing refers to flavour maturation. At the end of fermentation, many undesirable flavours and aromas of a “green” or immature beer are present (Bamforth, 2016). The ageing process reduces the levels of these undesirable compounds to produce a mature product: stable, quality suitable for filtration and packaging. The purposes of ageing are chill haze formation, clarification, carbonation, flavour maturation and stored capacity for demand smoothing. The total diacetyl concentration is used to judge the maturity of fermenting beer and must be decreased below the flavour threshold by means of brewing technology (Eblinger, 2009). The diacetyl precursor 2- acetolactate is also called ‘potential diacetyl ’, because it transforms into free diacetyl only in the filtered, yeast- free beer and then cannot be broken down any further. In calculating the total diacetyl concentration, 2 - acetolactate must be added to the amount of free diacetyl.

After ageing, clarification is required to remove any remaining yeast and suspended particles formed during cold storage. At least one filtration step is needed before the beer is suitable for packaging if a clear, brilliant beer is desired (Eblinger, 2009; Kunze, 2004).

The flavour stability is enhanced by excluding oxygen from the beer. Using of carbon dioxide to pack tank and to transfer beer reduces the possibility of air pick up (Hardwick, 1995).

CHAPTER III

MATERIALS AND METHODS

3.1 Materials

3.1.1 Maize and barley variety

The maize, Suwan 5, was purchased from National Corn and Sorghum Research Center (Thailand) and kept in a plastic bag until use. Barley malt for brewing was Pilsner malt (Weyermann, Germany) and was purchased from Rowatthai Co., Ltd, Thailand.

3.1.2 Microorganism

The Lager strain *Saflager* S-189 for bottom fermentation which is widely used in the brewing industry was purchased from Fermentis, France.

3.1.3 Hops

The Citra Hops pellets type 90 (10 – 14% acid) were used and purchased from YCH Hops, USA.

3.2 The influences of steeping duration and temperature on the α-amylase, β-amylase and protease activities of maize

3.2.1 Maize qualities

Proximate analysis of maize including moisture content, fibre content, total nitrogen content, fat content, ash content and available carbohydrate were analyzed according to AOAC (2000). Thousand grain weight and germination percentage of maize were determined according to European Brewing Convention (EBC). Gelatinization temperature of maize was carried out by Differential Scanning Calorimetry (DSC).

3.2.2 Experimental design

The steeping durations and germination temperatures were performed by full factorial design (1x3x5), three steeping durations (24, 48 and 72 h) and five temperatures (20, 25, 30, 35 and 40^{o}C). The experiments were duplicated.

3.2.3 Malting process

Five hundred grams of cleaned rough maize were weighed and put on aluminium mesh box. All mesh boxes were placed on the water bath and filled with RO water. Steeping water was replaced every 12 h with equilibrated temperature RO water in order to eliminate any inhibitor solubilized from grain and for circulation of oxygen to grain. This stage was called air-rest stage and all treatments were re-steeped

for 5 min in every 12 h. After steeping stage, maize was germinated up to 7 days. The temperature and relative humidity of germination step were controlled at 30°C and 95 ± 5%, respectively. The germinating maize sample from each treatment was taken once a day and kilned at 50°C for 24 hours. The dried sample was kept at -20ºC until needed for further analysis.

Enzyme activities of malt including α- and β- amylases, protease; steep-out moisture, malting loss, reducing sugar, shoot and root length were determined.

3.3 Influences of germination temperatures on malt quality

The malting process was manipulated by using 800 g of maize grain in metal boxes. All boxes of maize were washed with RO water before steeping at 30ºC for 24 h, 36 h and 48 h. After this equilibration, maize boxes were put in germination room with a relative humidity of 95±5% and controlled the temperature at 20, 25, 30, 35 and 40°C, separately. The steeping degree of maize was controlled by re-steeping or spray water. After the germination, the sprouted maize was kilned in a hot air oven. Root and shoot were removed.

Qualities of maize malt including malting loss, free amino nitrogen (FAN), soluble nitrogen, extract content, protease activity, α-amylase and β- amylase activities were determined according to EBC.

3.4 Optimization of the ratio of maize malt and barley malt for new larger beer

3.4.1 Effect of barley malt and maize malt ratio on wort qualities

Eleven types of wort were prepared at differences ratio of barley malt and maize malt. The reference samples were wort and beer obtained from 100% malt mashes. These were compared with the mixed worts produced from mixtures of malt and maize malt in the ratio 10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20, 90:10 and 100% of maize malt. Milled malt was mashed with distilled water as ratio 1:5 in the laboratory mashing bath. The wort qualities were determined.

3.4.2 Mashing and boiling

3.4.2.1 All barley malt wort production

The ground barley malt was mixed with water at 40°C. The mashing pH was adjusted as 5.0 to 5.2 by using lactic acid 1 M. Mashing-in was taken place at 45°C within 30 min. Next, the temperature was increased to 62°C within 20 min, followed by a 30 min rest; then 72°C within 20 min, followed by 30 min (Figure 3.1). Mash was transferred to the lauter tun, after separation of spent grains and the wort, the wort was boiled for 60 min at 100°C.

3.4.2.2 Supplemented maize malt wort production

Due to the gelatinization temperature of the maize starch being

higher than that of barley, the mashing process was chosen and modified following the method from Zweytick et al. (2005) and Poreda et al. (2013). The ground malt was suspended in water at 45°C. The pH value was adjusted using 1 M lactic acid.

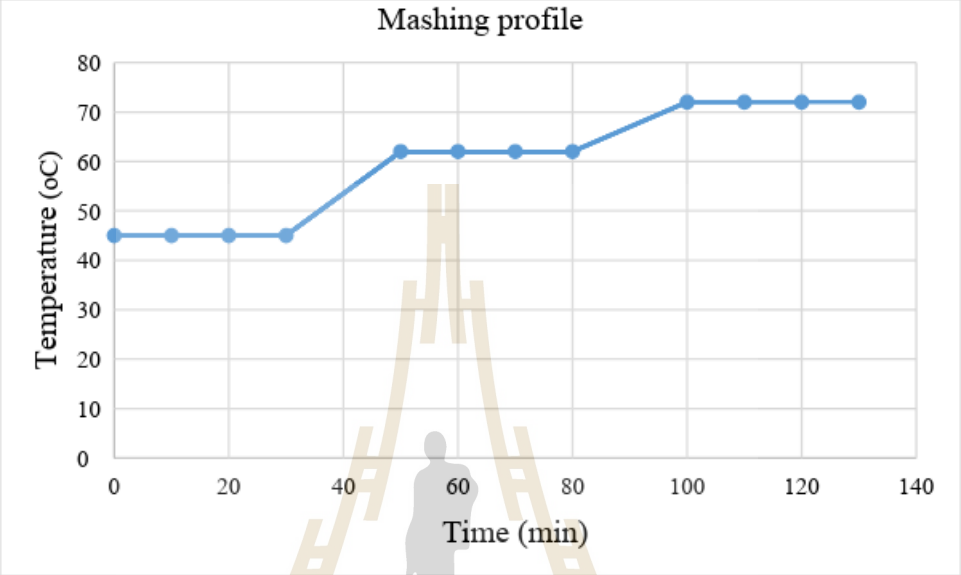

Figure 3.1 Mashing profile for all barley malt wort production.

First, an appropriate amount of maize malt grist with 10% of the ground barley malt was mixed with water, and mashing-in started at 45°C. The grist-to-water ratio of 1:5 was held at 45°C for 30 min. After that, the temperature of the mixed mash was increased in the cereal cooker to 62°C (which was taken 10 min) and held for 30 min. Then, the temperature was 72°C within 10 min and held for 30 min. Following this, it was increased to 100°C (20 min). When the boiling point was achieved, the mash from cereal cooker was transferred back to the mash tun, where the remaining portion of barley malt mash was held at 45°C for 30 min. On the completion of the transfer, the resulting mash had a temperature of 62°C (duration of the transfer was 10 min). This temperature was maintained for 30 min. After that, the temperature

was increased (10 min) to 72°C and maintained for 30 min (Figure 3.2). Finally, the whole portion of mash was transferred to the lauter tun. The obtained wort was boiled for 60 min – three quarters of the hop pellets were added after 15 min, one quarter after 45 min. The expected bitterness unit of wort was 30 BU. After cooling the wort down to 14°C, yeast added and the beer was left for fermentation.

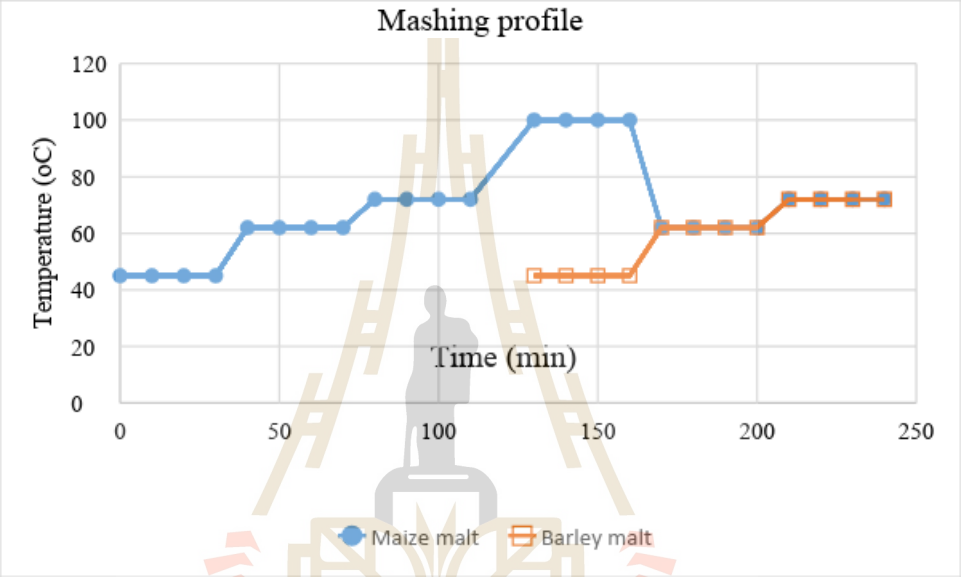

Figure 3.2 The decoction mashing profile for supplemented maize malt of barley wort production.

3.5 Beer fermentation

3.5.1 Inoculum preparation

The yeast strain *Saflager* S-189 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 cells were separated by centrifugation at 4,000 rpm for 10 min at 4°C 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 a microscope and calculated the volume of wort starter which needed to inoculated at 1.4×10^7 cells/mL of fermented wort.

3.5.2 Fermentation conditions

The suitable mashing methods were selected to study the fermentation and beer production. The wort was prepared as described in 3.4.2. The wort was sterilized by boiling at 100°C for 60 min and hops pellet was added to obtain final beer bitterness for 30 BU (mg of bitter substance per litre of wort) and further cooled to room temperature. Sterilized wort was transferred to the whirlpool for clarification and the wort was cooled to 14°C. Twelve litres of cast wort were transferred into 16 L of the sterilized fermenter. Bottom-fermenting yeast was pitched at 1.4×10^7 cells/mL at 14°C. Fermented wort was collected every 12 h for pH, viable cell count, FAN, ethanol, and reducing sugar analyses. The fermentable sugar content of fermented wort including glucose, fructose, maltose, and maltotriose concentrations was analyzed. Fermentation was stopped when the extracted content was unchanged within 48 h, the percentage of FAN and reducing sugar utilization were determined.

Maturation step was carried out at 4°C for 1 week. Residual yeast was eliminated by centrifugation at 4,000 rpm for 10 min and 4°C and carbonation of

finished beer was taken place by direct addition of CO_2 into a keg of beer and incubated at 4°C for 7 days. Physical properties of beer including beer colour, sensory analysis, and volatile compounds including alcohols, esters, acetaldehyde, DMS, and diacetyl of beer were analyzed.

3.6 Analysis

3.6.1 Analysis of malt qualities

3.6.1.1 Determination of steep-out moisture content

The steep-out moisture content was determined by putting all maize grains up on the filter paper for 5 min and weighed 5 g ground maize into moisture can. The sample was dried at 105°C for 3 h and calculated for the moisture content of grain according to the EBC (Enari, 1975).

3.6.1.2 α-amylase and β-amylase assays

The crude amylase enzyme was extracted by using 1 g of finely ground malt with 9 mL of 50 mM Tris-HCl pH 7.4 with 3 mM $CaCl_2$ and 4 mM NaOH and incubated at 25ºC for 30 min by shaking for 1 min in every 15 min. Then, the crude enzyme was separated by filtering it through cotton and centrifuging at 5,000 rpm for 10 min and kept on ice until needed. Starch hydrolysis by amylase enzymes was quantified by measuring reducing sugar which is the product of enzyme activities (Usansa et al., 2009). The 0.5 mL of crude α-amylase enzyme was incubated at 70°C for 5 min, then 0.5 mL of 1% (w/v) soluble starch containing 50 mM acetate buffer

(pH 5.5) with 0.003% $CaCl_2$ was added. The reaction was continued for 10 min and terminated by addition of 1 mL of 3-5, dinitrosalisylic acid reagent. For β-amylase assay, the reaction was constructed in the same procedure as α-amylase but crude enzyme was incubated at 55°C, and 1% (w/v) of soluble starch containing 50 mM citrate buffer (pH 3.6) with 1 mM EDTA was used as the substrate of reaction.

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

3.6.1.3 Protease assay

The protein assay was carried out according to Bradford's method (Bradford, 1976). The protein content of crude extract is measured base on the addition of protein to a Coomassie Blue G-250 dye. In the presence of the protein the Bradford solution will change the colour from brown to blue. The intensity of this colour change is dependent upon the protein content of the sample – the higher the protein concentration the more intense the colour change. This colour change is measured by Spectrophotometer at a wave length of 595 nm and from this the protein content of the sample can be determined using a calibration curve produced from known concentrations of BSA (Bovine Serum Albumin) as a standard.

In the present study, 50 µL of crude extract was added to 3 mL of Bradford reagent, incubated at room temperature at least 5 minutes. The intensity of the colour change read using Spectrophotometer. The resultant absorbance readings were used to calculate the protein content of the crude extract using calibration.

3.6.1.4 Protease activity

The protease activity of crude extracts was assayed using the protease substrate azocasein. Azocasein comprises of the protein casein derivatised with an azo dye. Proteolytic cleavage of azocasein discharges free azo dye into the surrounding solution, in this way changing its colour. The amount of dye released is directly proportional to the amount of protease activity present in the sample and can be measured by spectrophotometer at an absorbance wavelength of 440 nm.

Table 3.1 Assay buffer systems

Reagen	Buffer
A	0.50 %(w/v) Sodium bicarbonate buffer ($NaHCO_3$), pH 8.0 (Prepare 100 ml in deionized water using Sodium bicarbonate. Adjust to pH 8.0 with 1M HCl.)
B	2.50 %(w/v) Azocasein solution (Prepare 15 mL in reagent A. Gentle heating and stirring may be needed to from a solution.)
C	5.0 %(v/v) Trichloroacetic acid solution (TCA) in DI water.

The method consists in mixing equal volumes of the substrate and enzymatic sample at a given temperature and pH that corresponds to the optimum conditions of the enzyme under investigation. The protease activity was determined follow the below protocol.

The crude extracts were assayed using the pH values and buffers listed in Table 3.1. All assays were accomplish in 1.5 mL Eppendorf tube at 37^{o}C in a water bath.

Assays were carried out by adding 100 µL of crude extract with 150 µL reagent A and 250 µL Azocasein solution. The assays were then shaken well to ensure thorough mixing and incubated in a constant temperature water bath at 37°C for 30 minutes. Controlled consisted of 250 µL reagent A and 250 µL Azocasein solution. Assays were stopped by the addition of 1 mL of 5% TCA, shaken and left in room temperature for 10 minutes to ensure full precipitation of any undigested Azocasein. The assays were then centrifuged at 10 000 rpm for 5 minutes to yield an yellow-orange supernatant containing the cleaved azo dye. After centrifugation 1 mL of supernatant from each assay was transferred to 1 mL cuvette and analyzed spectrophotometrically at the absorbance wavelength of 440 nm.

3.6.1.5 Malting loss

The weight loss of germinated grain as a consequence of germination process was calculated as a percentage and expressed on a wet weight basis. One hundred dried grains were counted by manual.

$$\% \text{ Malting loss} = \frac{(\text{Weight of 100 corn grains} - \text{Weight of 100 malted corn}) \times 100}{\text{Weight of 100 corn grains}}$$

3.6.1.6 Shoot and root length

Shoot/root length of germinated grain was determined by sampling 10 grains from each treatment and measuring them with vernier caliper. The results were the average values from a duplicate set of rice malt samples.

3.6.1.7 Reducing sugar

The amount of reducing sugar from germinated maize was analyzed according to Miller (1951) by weighing 1 g of maize grain and grinding it to fine particle. One gram of powdered maize and 10 mL of distilled water was added and incubated at 20ºC for 30 min. Then, the solubilized reducing sugar was separated by centrifuging at 5 000 rpm for 10 min. The 0.5 mL of supernatant was taken for mixing with 0.5 mL of 3,5-dinitrosalicylic acid solution in the 16 mL test tube. The development of colour was conducted by boiling the reaction tube for 10 minutes. The concentration of reducing sugar was calculated against the standard of maltose concentration 0.2-1.0 µmole/mL.

3.6.1.8 Extract of malt: Congress malt

Extract of maize malt was performed according to EBC 4.5.1 (1998). Fifty grams of ground malt was mixed with 200 mL of water and stirred at 100 rpm in mashing water bath until mixture in the mash reaches 45°C. The mixture was maintained the temperature of 45ºC for exactly 30 min. Then, the temperature of mash was raised 1ºC per min for 25 min. When the temperature reached to 70ºC, 100 mL of water at 70ºC was added and incubated for 60 min. The mashed 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 by hydrometer at 20ºC. Extract content of wort was calculated according to the formula:

$$E_1 = \frac{P(M+800)}{100-P}$$

$$E_2 = \frac{E_1 \times 100}{100\text{-}M}$$

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 (^oP)

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

3.6.1.9 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 colour 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 (107.2 mg glycine in 100 mL water and diluted to 1:100 before used). The FAN content in malt was calculated using the formula:

$$\text{FAN} = \frac{\text{OD of test solution}}{\text{Mean OD of standard}} \times 2 \times \text{dilution factor}$$

3.6.2 Analysis of wort and beer

3.6.2.1 Reducing sugar of wort

The amount of reducing sugar from fermented wort was analyzed according to Miller (1951) as mention in section 3.6.1.7.

3.6.2.2 Free amino nitrogen (FAN) of wort

Free amino nitrogen of wort and beer was 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 colour reagent was added. The 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 ($mg.L^{-1}$) was calculated the using formula:

$$FAN = \frac{A_1 \times 2d}{A_2}$$

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

3.6.2.3 Extract content of wort

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

$$E_1 = \frac{P \times (M+W)}{100 - P}$$

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)

3.6.2.4 Fermentable sugar and ethanol in wort and beer

Fermentable sugars in the 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 was prepared. Samples were diluted with DI water at ratio 1:50 and filtered through 0.22 µm filter paper before analysis. Ten µL of the sample was injected through a Rezex ROA-Organic Acid column 300 x 7.8 mm (Phenomenex, U.S.A). The temperature of

column and detector were set at 55ºC and 35ºC, respectively. The quantity of fermentable sugar and ethanol in samples were calculated using standard equation.

3.6.2.5. 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 Agilent 7890A coupled with a mass detector model 7000 Triple Quad GC/MS (Agilent, U.S.A)) by solid phase microextraction (SPME) technique. Samples were heated to 70ºC and agitated continuously at 600 rpm for 20 min. The volatile compounds of samples were collected using Polydimethylsiloxane (PDMS) fibre syringe (Supelco, U.S.A).

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

The samples and standard were injected and separated on a DBWAX column, 60 m x 0.25 mm I.D. and 0.25 µm film thickness (Agilent, U.S.A.). Column temperature-program was 35ºC for 5 min and increased to 230ºC by 6ºC per min (running time 42.5 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 spectral 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 (10, 20, 40, 60, 80 and 100 ppm) including dimethyl sulfide (DMS), acetaldehyde, propanol, isoamyl alcohol, butanol, ethyl acetate, isoamyl acetate, 2,3-butadione, 2,3-pentadione, 2,3-hexadione, 2-phenylethyl acetate, octanoic acid, ethyl caproate, ethyl caprate and ethyl caprylate were prepared using 40% ethanol as a diluted solution.

3.6.2.6 Viable cell count

Viable cell count of yeast cell in fermented wort was measured 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 a microscope.

3.6.2.7 Beer foam stability

The foam stability was measured as the seconds needed for the foam to collapse over a distance of 30 mm. The measurement starts 10 mm below the rim of the glass. The time periods it takes for the foam to collapse over the distances of 10, 20 and 30 mm were displayed as a result.

3.6.2.8 Colour of beer

Colour of beer was measured by Spectrophotometric method which determined the absorbance of diluted sample at 430 nm within the linearity of the spectrophotometer. The colour for sample was calculated according to EBC8.5 (1998) using the formula:

$$\text{Colour (EBC units)} = A.\ f.\ 25$$

Where:

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

f = dilution factor

3.6.2.9 Sensory analyses of beer

A sensory evaluation of the beers was carried out by a trained, 16-member tasting panel (women and men, 20 to 60 years old), following the EBC method (Analityca, 2007). Sensory tests of beers were performed using Hedonic Test. The five attributes assessed for the taste were appearance, aroma, flavour, mouth-feel and overall-impression. The hedonic scale (5 levels) was designed as 0 to 5 scores for evaluating the aroma, appearance, flavour and mouth-feel. The score 0 was extremely dislike, 1 was dislike, 2 was normal, 3 was like, 4 was like very much, 5 was extremely like. For an 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.

3.6.3 Statistical analysis

The statistical analysis was carried out by using SPSS version 20.0 for Windows. All chemical experiments were analyzed in duplicates. 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 DISCUSSION

4.1 Maize properties

The proximate compositions, thousand grain weight and percentage of germination of maize *(Zea mays)* used in this study were analyzed. These properties were essential parameters for accomplishing the homogenous quality malt.

Suwan 5 is the single cross hybrid maize, a source for high-yielding and downy mildew–resistant crops. It had good agronomic traits and also gave high fresh and dry weights (Aekatasanawan et al., 1994). The high-yielding property of this maize explains the very high percentage of germination (96%). Moreover, the thousand grain weight of maize was totally higher barley and other grains (wheat, rice and sorghum) due to the big size of the grain.

Maize contained 9.01% of protein which was approximate with barley (9 – 11%). As shown in Table 4.1, the carbohydrate content of maize was 73.87% which was similar to barley (75 – 79%). It was indicated that maize is a potential carbohydrate source for brewing. On the other hand, maize contained 3.9% of fat which higher than barley (2.0%). However, during the germination of maize, the fat content was decreased according to Ingle et al. (1964). The bulk of the fat reserve of the seed was progressively depleted over the 5-day germinated period from 10 mg/part to 3 mg/part.

These varieties of maize have benefits for malting and brewing from its properties.

Table 4.1 The proximate analysis result of Suwan 5 and Pilsner malt.

Nutrients	Chemical compositions	
	Maize (Suwan 5)	**Barley malt**
Moisture (%)	8.79 ± 0.09	5.00 ± 0.02
Crude protein (N=6.25) (%)	9.01 ± 0.14	10.50 ± 1.00
Fat (%)	3.90 ± 0.23	1.78 ± 0.17
Crude fiber (%)	2.04 ± 0.05	3.47 ± 0.41
Carbohydrate (%)	73.87 ± 1.36	76.24 ± 0.98
Ash (%)	2.17 ± 0.12	0.93 ± 0.09
Percentage of germination	96	ND
Thousand grain weight (g)	343.77 ± 4.09	42.07 ± 0.78

4.2 The influences of steeping duration and temperature on the α-amylase, β-amylase and protease activities of maize

4.2.1 Effect of steeping durations and temperatures on steep-out moisture

In order to determine the water uptake and the effect of steeping duration and temperature, maize was investigated at variously time 24, 48 and 72 h and differently temperature at 20, 25, 30, 35 and 40^{o}C. The results of steep-out moisture were obtained and shown in Table 4.2 and Figure 4.1.

Generally, when the maize was steeped in water, the moisture content was extremely rapid at the beginning but progressively slowed down after 24 h. At this early stage of water uptake, the moisture content increased from 8.79 to 32.81%, 36.56 and

38.52% at 20, 25 and 30°C, respectively. Thereafter, the moisture content slightly increased and continued at a linear until it reached its saturation point. After 72 h of steeping, maize reached a moisture content of approximately 42%. The statistical analysis of variance demonstrates that the steeping time significantly affected steep-out moisture (Table 4.2). Brenda et al. (2014) reported that steeping time had a significant effect on water absorption of corn grits. The corn steeped showed its maximum absorption of water at 6 h, after this time there were no significant differences in moisture gain in the corn steeped at room temperature. These results also agreed with Wijngaard et al. (2005). The moisture content of barley increased during 13 h steeping, from 11.73% to 40.02%. At this point water uptake slowed and after 87 h of steeping barley reached a moisture content of 45.48%.

Many factors influence the water uptake of kernels, for instance, steeping time, steeping temperature, the initial moisture of grains. It can be seen from the graph, the steep-out moisture content was not only enhanced by steeping time but also by temperature. The steep-out moisture at 30, 35 and 40 °C was higher than 20 and 25 °C, respectively. The water uptake is heavily influenced by temperature. Typically, maize develops well in warm and humid conditions. The water absorption was increased by temperature. Steeping using warm water (30, 35 and 40°C) may caused adequate hydration of the starchy endosperm of the maize grain by greatly expanding the pores in the endosperm, hence causing it to imbibe more water than steeping with water at ambient water condition (20 and 25°C). No significant difference was observed between the moisture content of the grains steeped at 30, 35 and 40°C.

Table 4.2 The steep-out moisture content after steeping for 24, 48 and 72 h at various temperature.

Temperature (°C)	Steeping durations		
	24 h	**48 h**	**72 h**
20	32.81 ± 0.67^{a}	39.11 ± 0.77^{a}	41.31 ± 0.47^{a}
25	36.56 ± 0.96^{b}	40.90 ± 0.52^{b}	42.19 ± 0.39^{b}
30	38.52 ± 0.44^{c}	41.62 ± 0.41^{c}	42.49 ± 0.28^{b}
35	38.64 ± 0.52^{c}	41.84 ± 0.39^{bc}	42.51 ± 0.28^{b}
40	38.76 ± 0.79^{c}	41.83 ± 0.36^{bc}	42.76 ± 0.33^{b}

Mean values of four replication of analysis ± standard deviation. The different superscript letter between the rows was a significant difference at $p \leq 0.05$.

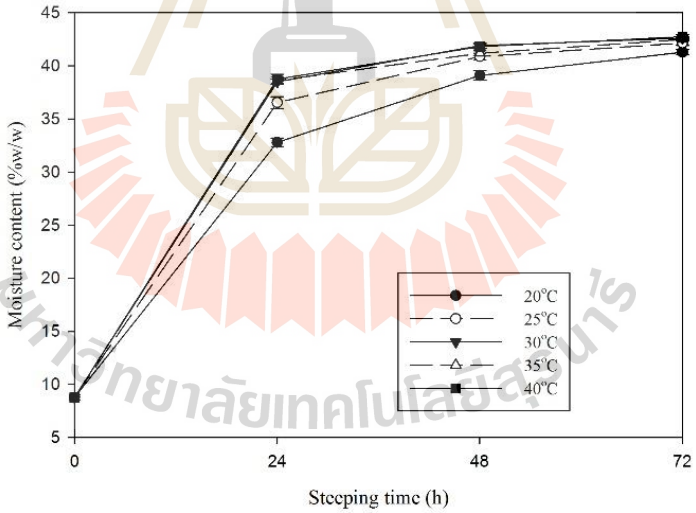

Figure 4.1 The steep-out moisture content of maize at various temperature. Error bar indicated the standard deviation of four measurements.

4.2.2 Effect of steeping durations and temperatures on amylases activities

In recent years, several researchers have done with their works showing

the optimum steeping conditions of various grains (Brenda et al., 2014; Ebbah et al., 2015; Eneje et al., 2004; Iwuoha & Aina, 1997; Montanuci et al., 2016; Usansa et al., 2009; Wijngaard et al., 2005). Amylase is an enzyme that catalyzes the hydrolysis of starch into sugars. Alpha-amylase is an endo-enzyme, which randomly acts on locations along the starch chain releasing small dextrins and fermentable sugars. Beta-amylase is an exo-enzyme that working from the non-reducing ends of amylose and amylopectin, resulting in release maltoses.

In this research, the α- and β-amylase activities of maize were examined during steeping time and up to seven days of germination. As is illustrated in Table 4.2, when steeping time increased from 24 to 72 h, the amylase activities of maize were enhanced. Through these time, the α-amylase has continuously risen. The maize steeped at 30^{o}C which corresponds which a steeping time of 72 h, showed the highest α-amylase activity (6.68 U/mg). In addition, comparison of β-amylase activity in steeped maize, the maximum β-amylase activity was lower. The largest β-amylase was also found in maize steeping at 30^{o}C for 72 h. The lowest α- and β-amylase activities were presented in maize steeping at 40^{o}C when compared which other samples. In the same way, Eneje et al. (2003) described the development of diastatic power among the steeping germination time of Nigerian white and yellow maize.

The α-amylase enzyme was formed during malting process, values were shown in Figure 4.2 (A, C, E). Low rates were observed during first 3 days of germination. After that, from day forth, the α-amylase activities rapidly increased to maximum value, from 9.78 U/mg to 31.10 U/mg and 10.98 U/mg to 35.76 U/mg of samples steeping at 25 and 30^{o}C, respectively. When the maximum amounts were reached, the enzyme activities gently decreased. The development of α-amylase was

higher in the hydration process at 25 and 30°C in the time of 24 h hydration. The sample steeped at 30°C for 24 h was the best sample for the enzyme development. This result was similar with study of Nadeem and colleagues (2017), when they maximum germination percentage (99%) was found in MMRI-yellow cultivar of Maize soaked at 24 hours of duration followed by 94% germination soaked at 36 hours, 87% germination at 12 hours soaking time and 69% germination rate at 0 hour soaking, respectively. Two samples with steeped at 35 and 40°C for 24, 48 and 72 h were not suitable for enzyme production, with slightly increased in the amount of enzyme in the process, with an initial was 2.5 U/mg and final value approximate 6 U/mg.

The β-amylase also presented in maize, with intensification or activation of its production in the malting process. The enzyme expression during germination was illustrated in Figure 4.2 (B, D, F). Similar with α-amylase, β-amylase was also significantly affected by steeping temperature and steeping time. However, the values of β-amylase were lower than α-amylase.

The α- and β-amylase values found in this study higher than those reported by Usansa et al. (2009), where the highest α- and β-amylase amounts of rice malt were approached 20 U/mg. In addition, the obtained results also greater than the study of Wijingaard (2005) from buckwheat malt.

Furthermore, the enzyme activity was also depended on steeping time. After long period soaking in water, the enzyme activity of maize decreased by time. Warm temperature caused by the fast rate of oxygen depletion and long steeping duration may cause anoxia condition to occur. Guglielminetti et al. (1995) demonstrated the activity of α-amylase and de-branching enzymes were decreased when germinated

in anoxia condition. Therefore, the lower reducing sugar in germinated maize from longer steeping condition also confirmed that the starch digestion was retarded by a long steeping duration (Figure 4.3). Many authors have mentioned that excessive steeping without aeration damage the germinative capacity of grains and leads to uneven growth (Brookes et al., 1976).

Table 4.3 The α- and β- amylase activities in the sample after steeping for 24, 48 and 72 h at five temperature 20, 25, 30, 35 and 40°C.

Temperature (°C)	α-amylase activity (U/mg)			β-amylase activity (U/mg)		
	24 h	48 h	72 h	24 h	48 h	72 h
20	2.77±0.30^{a}	3.32±0.09^{b}	4.46±0.11^{c}	2.52±0.10^{a}	3.45±0.26^{b}	4.23±0.00^{c}
25	3.06±0.08^{a}	3.69±0.25^{b}	4.60±0.09^{c}	2.86±0.10^{a}	3.63±0.23^{b}	5.36±0.29^{c}
30	3.72±0.10^{a}	5.25±0.14^{b}	6.68±0.11^{c}	3.32±0.10^{a}	3.89±0.17^{b}	5.42±0.28^{c}
35	2.62±0.03^{a}	3.18±0.04^{b}	3.26±0.07^{b}	2.25±0.16^{a}	3.75±0.10^{b}	3.76±0.17^{b}
40	2.78±0.03^{a}	3.18±0.03^{b}	3.24±0.08^{b}	2.51±0.14^{a}	3.29±0.00^{b}	3.40±0.07^{b}

Mean values of four replications of analysis ± standard deviation. The different superscript letter between the columns was a significant difference at $p \leq 0.05$.

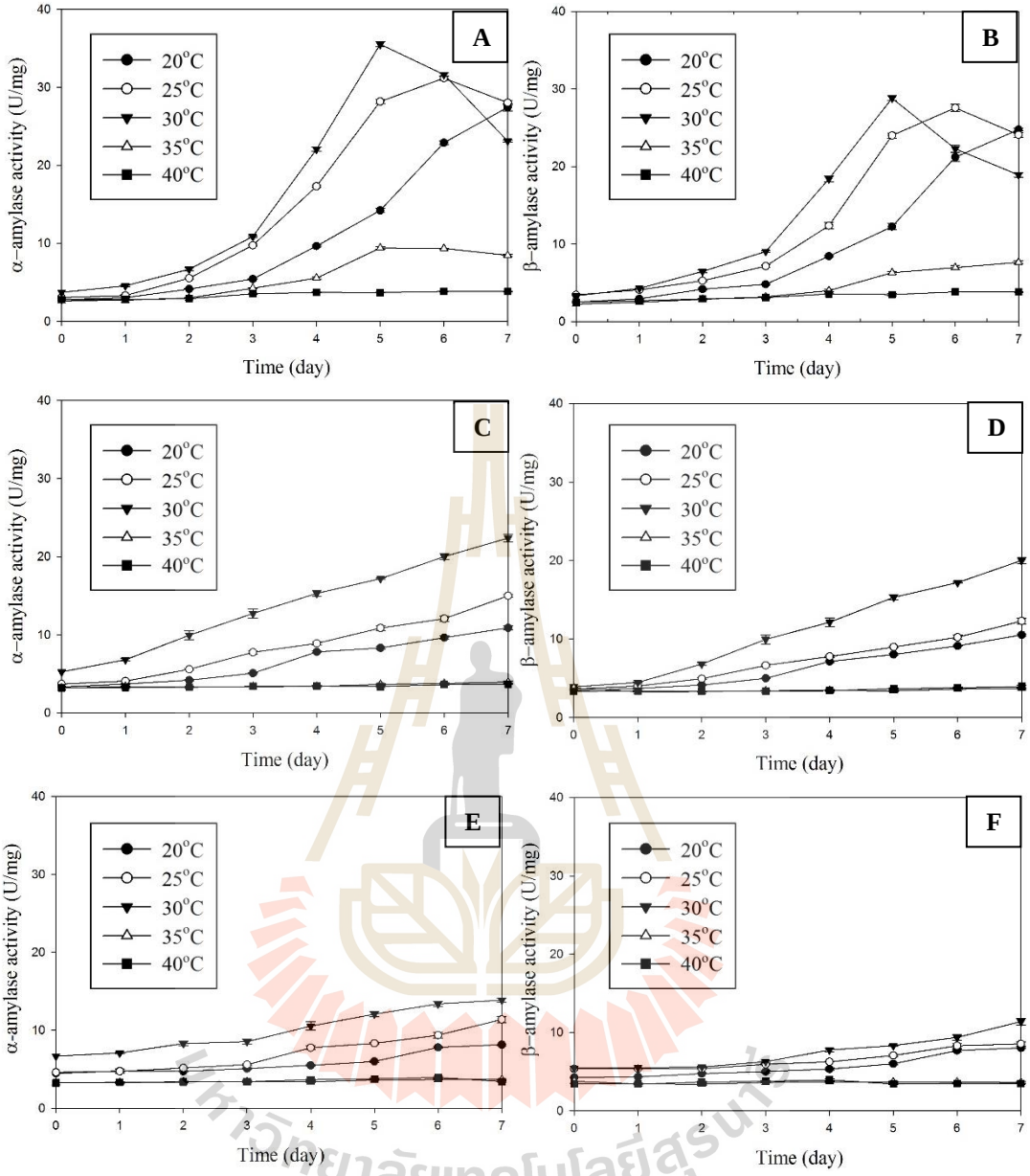

Figure 4.2 The time course of α- and β-amylase production in germinating maize at different steeping temperature and duration: A & B: 24 h, C & D: 48 h, E & F: 72 h. A, C & E: α-amylase activity. B, D & F: β-amylase activity. Germination temperature was at 30ºC.

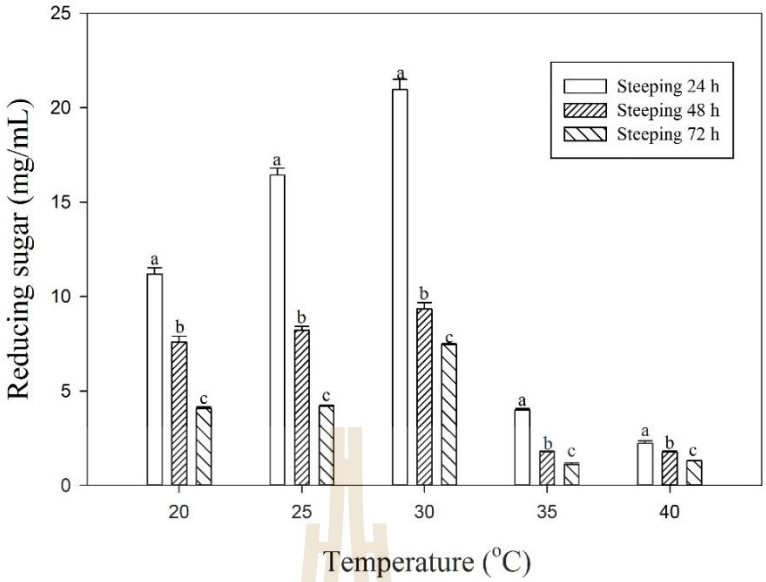

Figure 4.3 The reducing sugar of germinated rice after 7 days of germination time at different steeping duration. Error bar indicated the standard deviation of four measurements. The different letter between the columns was a significant difference at $p \leq 0.05$.

4.2.3 Effect of steeping durations and temperatures on protease activity

The protease activity of germinated maize was analyzed and shown in Figure 4.4. When the grain was malted and germinated, the overall protease activities were increased during 1 – 5 days of germination when the grains were steeped for 24 h and from day 1 – 7 which maize steeped for 48 and 72 h. The enzyme activity occurred in different steeping regime. The highest value was found in the sample which steeping at 30°C for 24 h and followed 25, 20, 35 and 40°C, respectively. The results also demonstrated the effect of steeping time on proteolytic activity of maize, when increasing steeping time, the enzyme activities reduced because of the influences of the anoxic condition.

Numerous studies of effects of malting conditions on the production of production of α-amylase and protease activity have been made (Ebbah et al., 2015; Eneje et al., 2004; Iwuoha & Aina, 1997; Montanuci et al., 2016). Variety and steeping time affected both carboxypeptidase and proteinase activities significantly ($P<0.001$) during germination (Ogbonna et al., 2004). It is generally believed that protease activity parallels the α-amylase activity of germinated grains (Fleming et al., 1959).

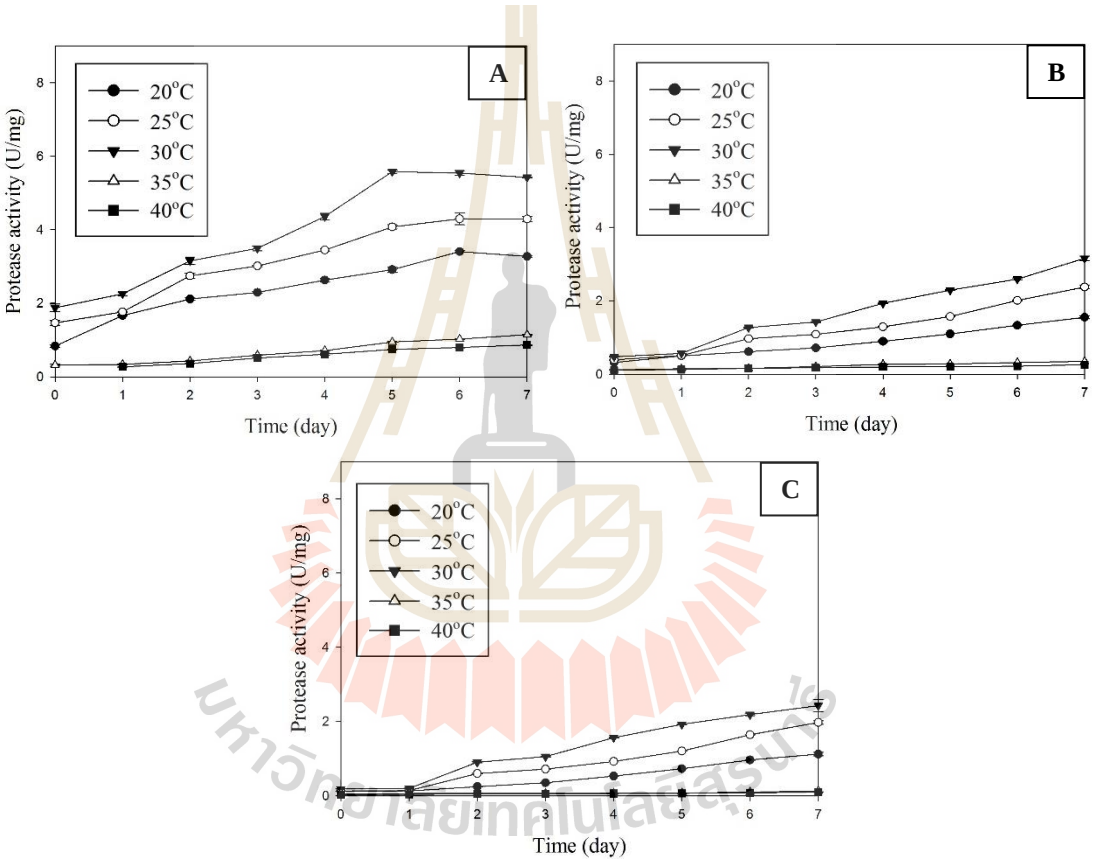

Figure 4.4 The time course of protease production in germinating maize at the different steeping duration: A: 24 h, B: 48 h, C: 72 h. Germination temperature was at 30°C.

4.2.4 Effect of steeping durations and temperatures on shoot/root formation and malting loss

After maize was steeped for 24 h, the growth of maize could be observed. One rootlet and single cotyledon pierced from the embryo and appeared outside the grain. At day forth, the presence of branch root could be observed and elongated until the end of germination. During malting, the elongation of shoot and root was slowly developed at first 3 days and progressively increased with germination time. However, the means of shoot/root length were compared for investigating the effect of steeping duration on growth development. The results indicated that the shortest of shoot/root length was found in maize steeped for 72 h, followed with 48 and 24 h. Whereas the temperature showed contrast effect, steeping and germination at 30^{o}C promoted longer shoot/root elongation than 25, 20, 35 and 40^{o}C, respectively. The malting loss was caused by metabolic activity during the germination and by the separation of shoot and root. Therefore, the value of malting loss increased with germination time and temperature. According to the meaning of malting losses, the steeping losses, rootlets losses and respiration losses were taken into account (Briggs, 1998). It was calculated as the reduction of dry weight and expressed as a percentage. From our results, malting losses of 72 h steeping duration were the lowest because the development of shoot and root was retarded. These results agreed with the studies of Agu and Palmer (1999) and Bekele et al. (2012) when they studied the effect of germination on Sorghum malt quality (Agu & Palmer, 1999; Bekele et al., 2012).

The results from these experiments suggested that the maize which steeped at 30^{o}C for 24 h was the best condition and chosen for further experiments.

Table 4.4 Shoot length, root length and percentage of malting loss of maize germination on 4th to 7th day.

Property	Time (days)	20°C			25°C			30°C			35°C			40°C		
		Steeping durations			Steeping durations			Steeping durations			Steeping durations			Steeping durations		
		24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
Shoot length (cm)	4	3.5±0.2^{a}	2.9±0.4^{b}	2.3±0.2^{c}	4.9±0.6^{a}	3.0±0.5^{b}	2.8±0.5^{c}	4.9±0.4^{a}	3.6±0.7^{b}	2.9±0.3^{b}	1.9±0.7	1.3±0.7	1.1±0.5	1.1±0.5	1.0±0.6	0.6±0.4
	5	5.0±0.3^{a}	3.5±0.4^{b}	3.1±0.6^{b}	5.1±0.3^{a}	3.7±0.6^{b}	3.0±0.7^{b}	5.6±0.5^{a}	3.9±0.6^{b}	3.0±0.3^{c}	2.4±0.7	1.5±0.7	1.4±0.7	1.4±0.5	1.3±0.7	1.1±0.7
	6	6.4±0.4^{a}	5.1±0.7^{b}	4.4±0.9^{ab}	5.9±0.5^{a}	3.9±0.7^{b}	3.2±0.6^{b}	5.9±0.5^{a}	4.0±0.6^{b}	3.1±0.4^{c}	2.7±0.6	1.9±0.7	1.7±1.1	1.9±0.7	1.8±1.0	1.8±1.0
	7	7.0±0.9^{a}	5.8±0.7^{b}	5.0±0.5^{b}	6.1±0.7^{a}	4.0±0.7^{b}	3.4±0.2^{b}	6.1±0.5^{a}	4.2±0.5^{b}	3.3±0.7^{c}	2.9±0.3	2.2±0.5	2.2±0.9	2.5±0.8	2.2±0.8	1.9±1.0
Root length (cm)	4	3.38±0.8^{a}	2.2±0.6^{b}	1.12±0.3^{c}	3.92±1.4^{a}	2.8±1.0^{ab}	2.1±0.4^{b}	4.9±0.4^{a}	3.6±0.7^{a}	2.9±0.3^{b}	2.2±0.7^{a}	1.8±0.8^{ab}	1.1±0.6^{b}	1.4±0.5	1.3±0.6	0.8±0.3
	5	4.1±1.1^{a}	2.5±0.6^{b}	1.5±0.1^{c}	4.2±0.5^{a}	2.9±0.9^{ab}	2.4±0.5^{b}	5.6±0.5^{a}	3.9±0.6^{b}	3.0±0.3^{c}	2.7±0.7	1.8±0.7	1.7±0.7	1.7±0.5	1.6±0.7	1.0±0.3
	6	4.8±0.9^{a}	2.8±0.7^{b}	1.9±0.4^{b}	4.8±1.3^{a}	3.1±0.8^{b}	2.6±0.5^{b}	5.9±0.5^{a}	4.0±0.6^{b}	3.1±0.4^{c}	3.0±0.6	2.2±0.7	2.0±1.1	2.2±0.7	2.1±0.9	1.3±0.4
	7	5.2±1.1^{a}	2.9±0.9^{b}	2.2±0.5^{b}	5.7±1.4^{a}	3.3±0.8^{b}	2.8±0.5^{b}	6.1±0.5^{a}	4.2±0.5^{b}	3.3±0.7^{c}	3.2±0.3^{a}	2.5±0.5^{ab}	2.1±0.9^{b}	2.8±0.8	2.5±0.8	1.8±0.5
Malting loss (%)	4	2.5±0.3^{a}	2.1±0.3^{a}	1.5±0.3^{b}	3.0±0.5^{a}	2.6±0.3^{ab}	2.3±0.3^{b}	4.1±0.4^{a}	2.9±0.2^{b}	2.8±0.6^{b}	2.1±0.2^{a}	1.8±0.6^{a}	1.0±0.4^{b}	1.8±0.6	1.1±0.3	0.9±0.5
	5	6.2±0.6^{a}	3.0±0.7^{b}	2.0±0.6^{c}	8.1±0.6^{a}	4.1±0.5^{b}	2.6±0.2^{c}	10.7±0.9^{a}	6.0±0.7^{b}	3.9±0.3^{c}	2.3±0.3^{a}	2.0±0.6^{a}	1.1±0.3^{b}	2.0±0.7	1.4±0.3	1.3±0.4
	6	9.0±0.6^{a}	3.6±0.5^{b}	2.4±0.6^{c}	13.2±1.1^{a}	6.3±0.6^{b}	3.3±0.4^{b}	14.9±1.1^{a}	6.7±0.5^{b}	5.1±0.2^{c}	2.4±0.3^{a}	2.3±0.5^{a}	1.4±0.4^{b}	2.3±0.3	1.8±0.5	1.5±0.3
	7	12.2±0.8^{a}	5.5±0.5^{b}	2.7±0.8^{c}	15.2±0.7^{a}	6.9±0.6^{b}	3.9±0.4^{c}	17.9±0.8^{a}	7.6±0.3^{b}	5.5±0.3^{c}	2.6±0.3^{a}	2.5±0.4^{a}	1.7±0.4^{b}	2.5±0.4	1.9±0.5	1.6±0.3

The mean comparison among the columns of 24, 48 and 72 h was performed separately on their temperature and properties. The different superscript letter was significantly different at $p \leq 0.05$.

4.3 The influences of germination temperatures on malt quality

Based on the enzyme activities, the maize steeped at 30°C showed highest values of α- and β-amylase and protease activity. To determine impacts of germination temperature and germination time to maize malt quality. Maize grains was steeped at 30°C for 24, 36 and 48 h. Then, the hydrated maize was germinated in controlled temperatures 20, 25, 30, 35 and 40°C. After germination, the sprouted maize was kilned in the hot air oven at 50°C for 24 h. Finally, the dried shoot and root were separated and the maize malts were kept at room temperature for malt quality determination: α- and β-amylase, protease, FAN, Kolbach index, extract content and reducing sugar.

4.3.1 The α- and β-amylase activities from crude enzyme extraction

During malting, hydrolytic enzymes, many of which are secreted by the scutellum and aleurone layer, accumulate in the starchy endosperm and catalyze the partial breakdown of its structural components (Briggs, 1998). Alpha and beta-amylases are the essential enzymes used in brewing to evaluate malt quality. The longer germination time led higher enzyme activity, as shown in Figure 4.5, enzyme activities were slightly increased from first 2 days, afterwards, the α- and β-amylases were dramatically developed to highest value. In this study, the maize steeped for 24 h displayed highest enzyme activity, and the maximum value was reached at day fifth at 30°C, followed 20 and 25°C. At high temperature (35 and 40°C), the enzyme activity was not significantly changed cause by the inhibition of germination at high temperature. Riley (1981) demonstrated that at the depression of the rate of protein synthesis in the embryos of several tropical hybrids imbibing at high temperature

correlated with their known temperature sensitivity. It was concluded that the protein synthesis is an especially temperature sensitive process in germinating maize embryos and that this is the principal reason for the sensitivity of germinating maize seeds to high temperature (Riley, 1981). The maximum level of α- and β-amylase activity detected in maize were 39.76 U/mg and 32.88 U/mg, respectively.

Furthermore, as was mentioned before, due to the anoxic condition, when the grains were steeped at long time, the maize was effected led to the reduction of germination rate along steeping time. The enzyme activity lowest when the maize was steeped for 2 days. When prolonging the steeping time, the maximum amylase activity was reached longer. For the 24 h steeped sample, the greatest amount was obtained at 5^{th} day, and 6^{th} and 7^{th} for 36 and 48 h, respectively.

4.3.2 Effect of malting conditions to protease activity

The protease activity of maize at different malting conditions were evaluated base on the hydrolysis of azocasein. The overall protease activity in grain increased during malting. Protease activity is the result of the activities of a very complex mixture of exo- and endopeptidases (Jone et al., 1993).

The changing in proteolytic activity was observed during malting. From the results in Figure 4.6, the protease activity was similar to amylase activity. The effect of malting conditions (steeping time, germination temperature and germination time) were confirmed again. The enzyme activity significant increased through germination time. It can be obtained from Figure 4.6, the protease activity with germinated at 35 and 40°C was lower than the rate of 20, 25 and 30°C. The low enzyme activity could be

mainly due to the inhibition of high temperature. High temperature (over 35^{o}C) for maize would be expected to retard the metabolic rate. In embryos of maize, heat shock markedly activated protein synthesis during early stages of germination, and heat shock proteins synthesis was induced simultaneously with the synthesis of the bulk of proteins produced by embryo tissues before heat shock. In addition, Azarkovich (2016) reported the effect of heat shock to protein synthesis in seed embryos of various grasses during seed development and germination. The consequence, the physiology of seed was significance changed. He concluded that this could be a manifestation of additional molecular mechanisms improving embryo tolerance to unfavourable environmental conditions and as a result, their viability.

The highest amount was found in sample germinated at 30^{o}C. However, base on the steeping time and germination, the proteolytic activity was changed. This result indicated that, at day five of germination, the maize steeped for 24 h was suitable for enzymatic production.

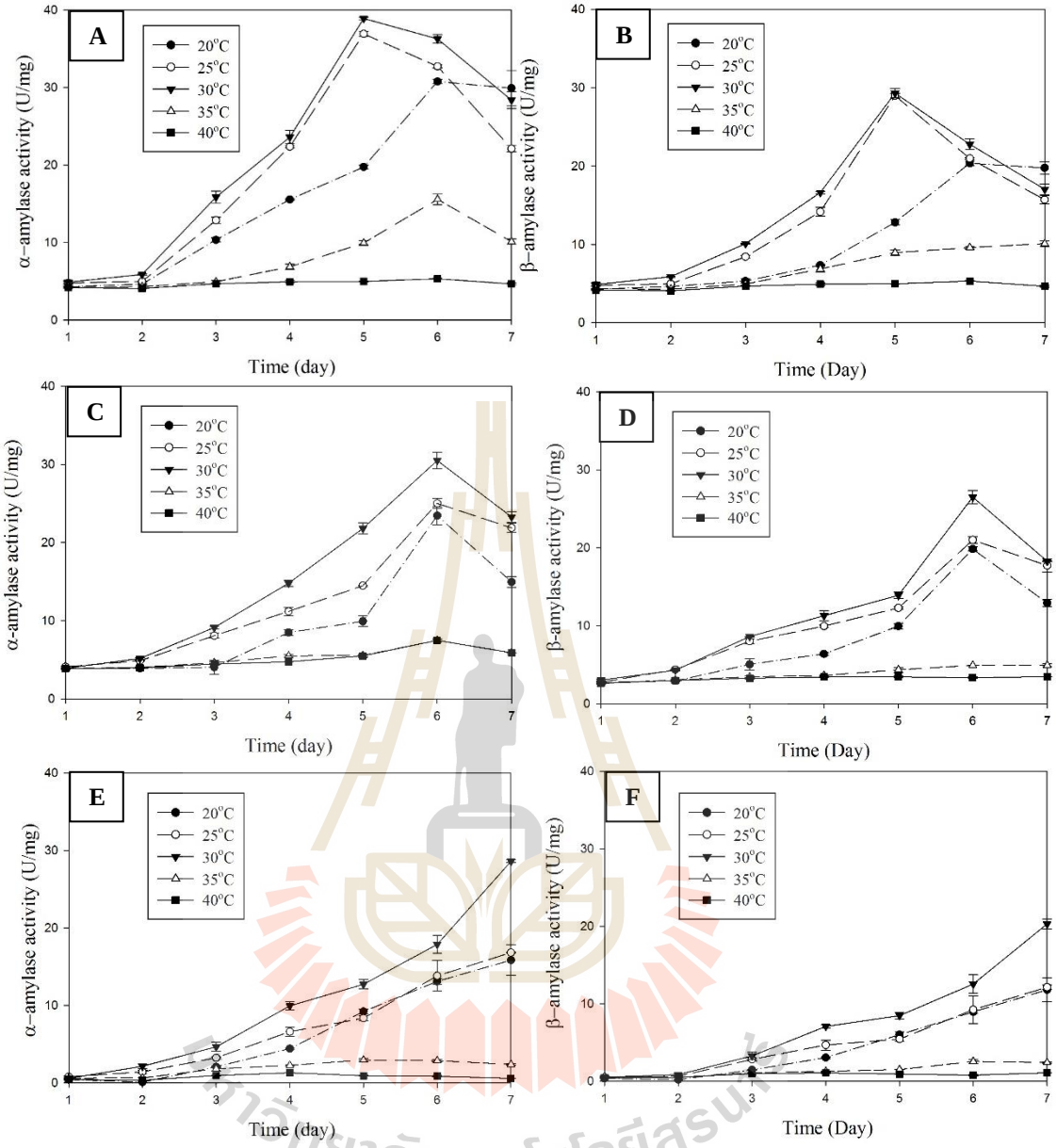

Figure 4.5 The time course of α- and β-amylase production in germinated maize at the different temperature: A & B: steeping 24 h, C & D: steeping 36 h, E & F: steeping 48 h. A, C & E: α-amylase activity. B, D & F: β-amylase activity. Steeping temperature was at 30°C.

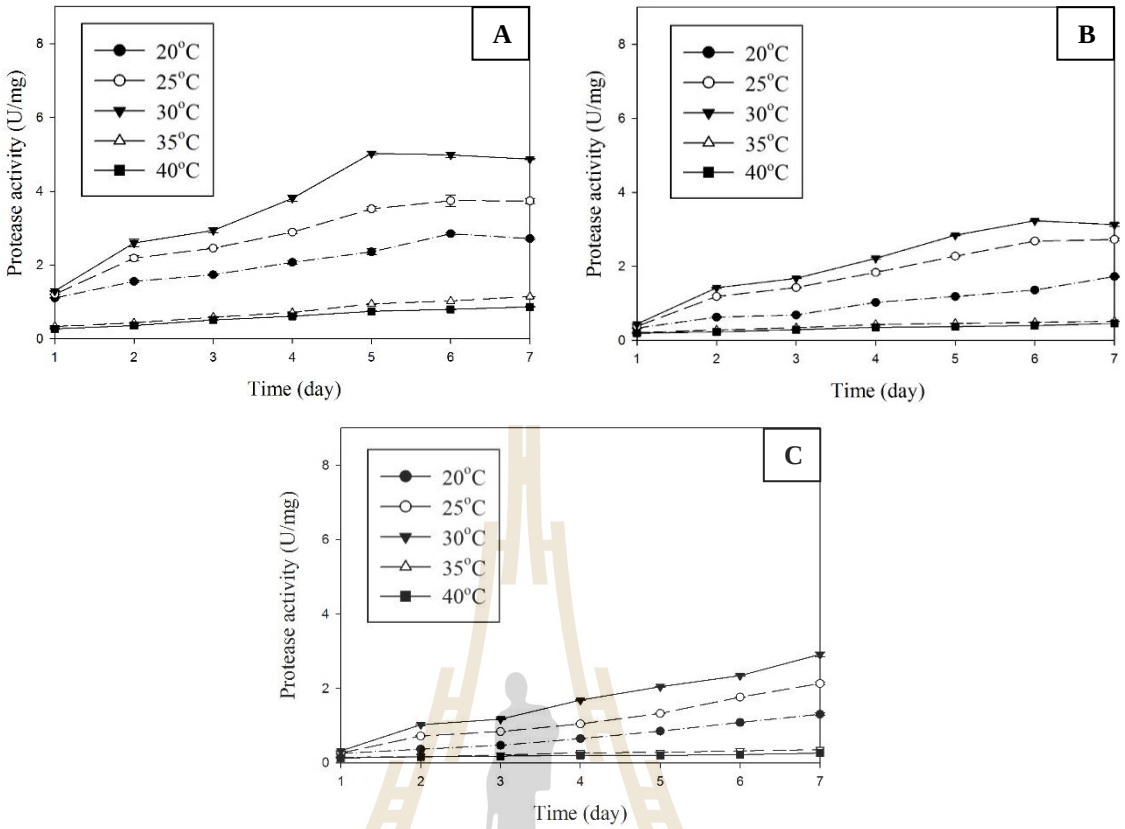

Figure 4.6 The time course protease activity in germinated maize at the different temperature: A: steeping 24 h, B: steeping 36 h, C: steeping 48h. Steeping temperature was at 30°C.

4.3.3 Nitrogenous substances

The nitrogenous components of malt are the most importance and have been widely studied (Agu & Palmer, 2001; Garde-Cerdán & Ancín-Azpilicueta, 2008). Nitrogenous compounds that are derived from the malt by proteolysis and extraction can affect fermentation, foam, mouth feel, the tendency to form hazes in the final beer and mash filtration. When grain is malted, the biosynthesis of many new nitrogenous fractions in the aleurone layer, gibberellic acid stimulates synthesis of hydrolytic enzymes, which are released into endosperm and hydrolyze enzymes.

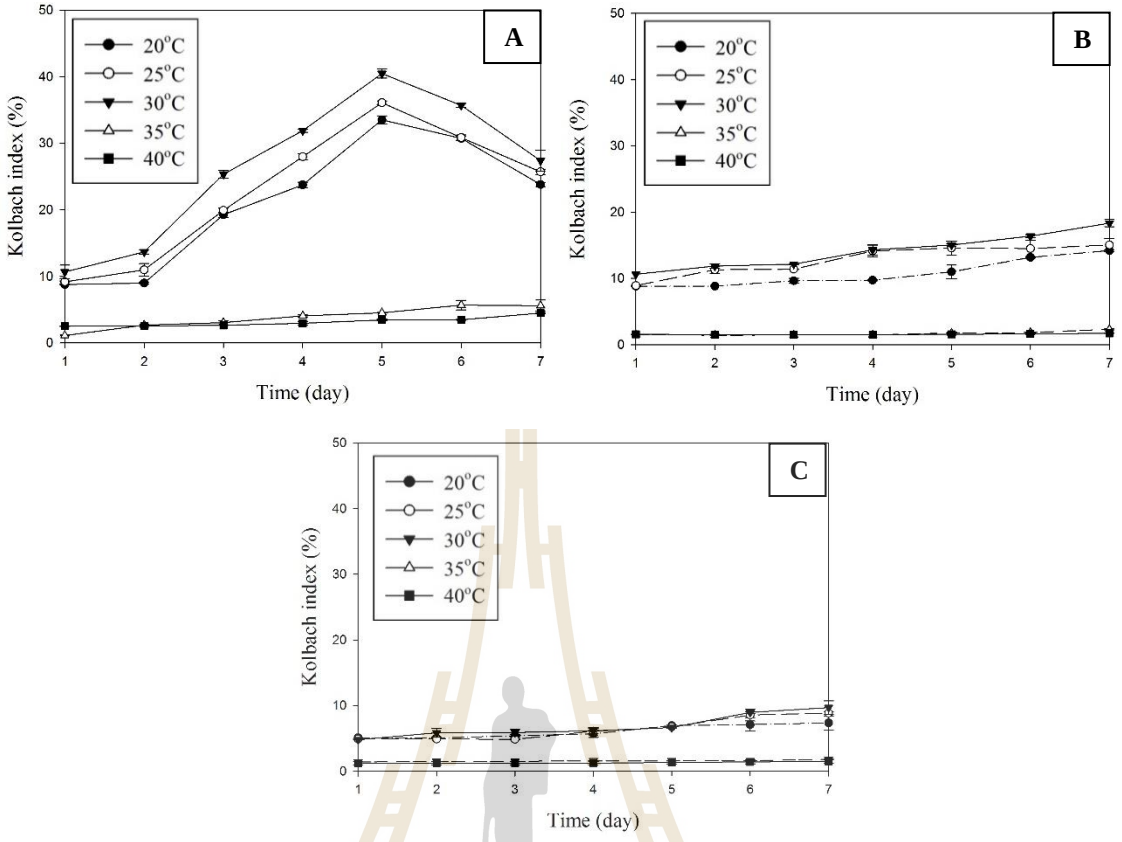

Figure 4.7 The time course of Kolbach index in germinated maize at the different temperature: A: steeping 24 h, B: steeping 36 h, C: steeping 48h. Steeping temperature was at 30°C.

The relation of the soluble nitrogen percentage on the total nitrogen percentage in term of Kolbach Index was analyzed. As can be observed in Figure 4.7, the Kolbach index was increased during germination time. The results indicated that the Kolbach index and the FAN were increased by increasing germination time and germination temperature (Figure 4.8). Two these parameters changed along the development of enzymatic activity, especially protease activity. The steeping regime for 24 h and germinated at 30°C at the fifth day germination provided the highest amount

of those nitrogenous components in rice malt. The FAN content in congress mash is confirmed to be a good index for potential yeast growth and fermentation efficiency. The FAN obtained from the maize malt in range 200 – 250 mg/mL. Therefore, the maize malt was qualified to be used in the beer production.

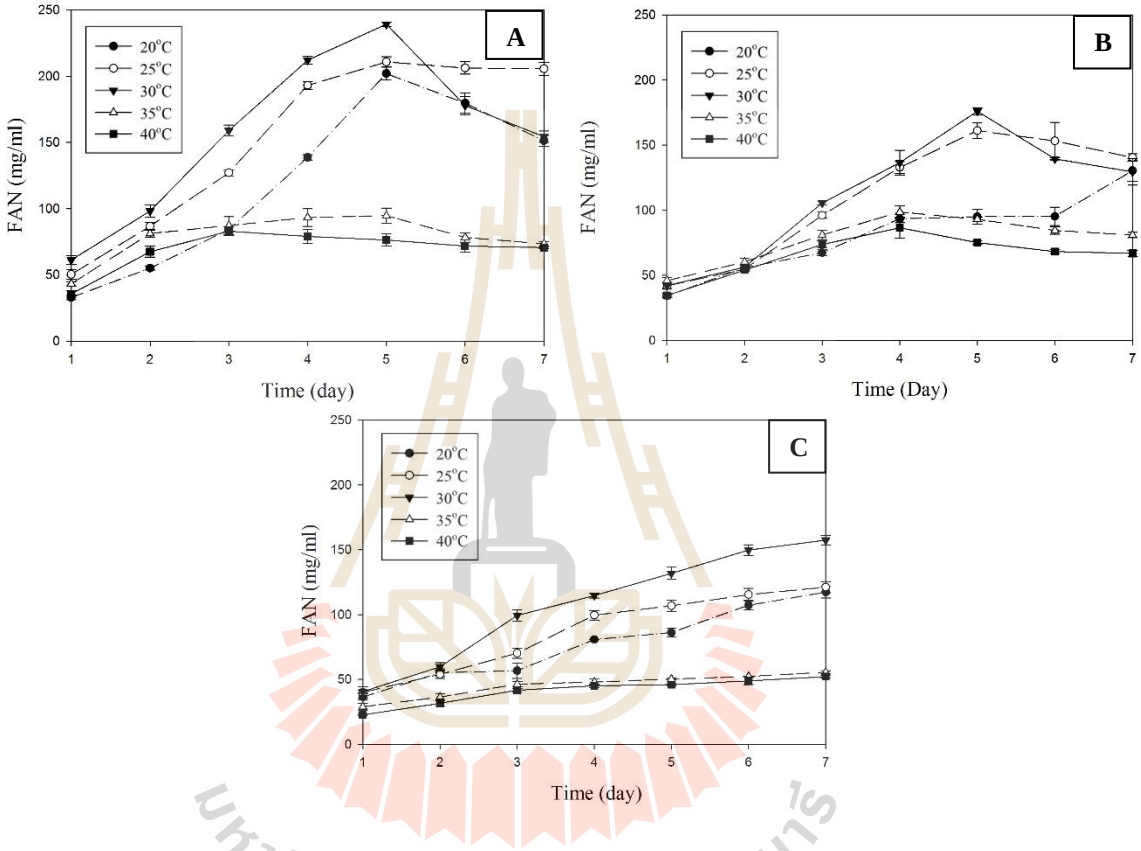

Figure 4.8 The time course of FAN in germinated maize at the different temperature: A: steeping 24 h, B: steeping 36 h, C: steeping 48h. Steeping temperature was at 30°C.

4.3.4 Extract content from congress mashing

Extract content is one of the most important parameters to determine malt quality and can be obtained from standard mashing program (EBC). In mashing, the

enzymes in malt (amylase and protease) break down insoluble substances of grain into soluble components. The extract content, therefore, present the yield of mashing in a percentage of dry matter.

Figure 4.9 compared five different germination temperature in terms of the extract content between 1st and 7th day of germination time. The results showed that through the germination time, the extract content was increased from the first day to seventh day of germination. At 24 h steeping time, the extract content presented the highest value, whereas, at 36 and 48 h steeping times, it was lower value of extract content. Since the development of amylase enzymes during germination at differential steeping time, the results explained the influences of enzyme activity to the concentration of extract. The increasing of enzyme activity correlates with the increasing of extract content. Hence, the steeping regime is important for the modification of maize during the malting process.

Moreover, the extract was significantly affected by germination time; particularly, at 24 h steeping time, the extract yield was increased with germination time if the temperature was less than 30°C. Once the temperature was increased to 35 and 40°C the extract yield was vigorously lower than that of 30°C. The highest value of extract from maize malt was 61.5%; while the extract of barley in range 79.3 to 81.4% (EBC). The explanation for this lower extract yield is the gelatinization temperature of maize starch was higher than 72°C (the gelatinization temperature of maize was 75.6°C), the saccharification temperature in congress mashing. This obtained results similar with report of Pelembe et al. (2004), the low extract content of sorghum malt caused by the starch gelatinization and hydrolysis. Thus, the low amount of extract content found in maize malt was reasonable and acceptable.

In addition, the concentration of reducing sugar presented in congress wort corresponded with the extract yield was logically increased with germination time and affected by germination temperature as depicted in Figure 4.10.

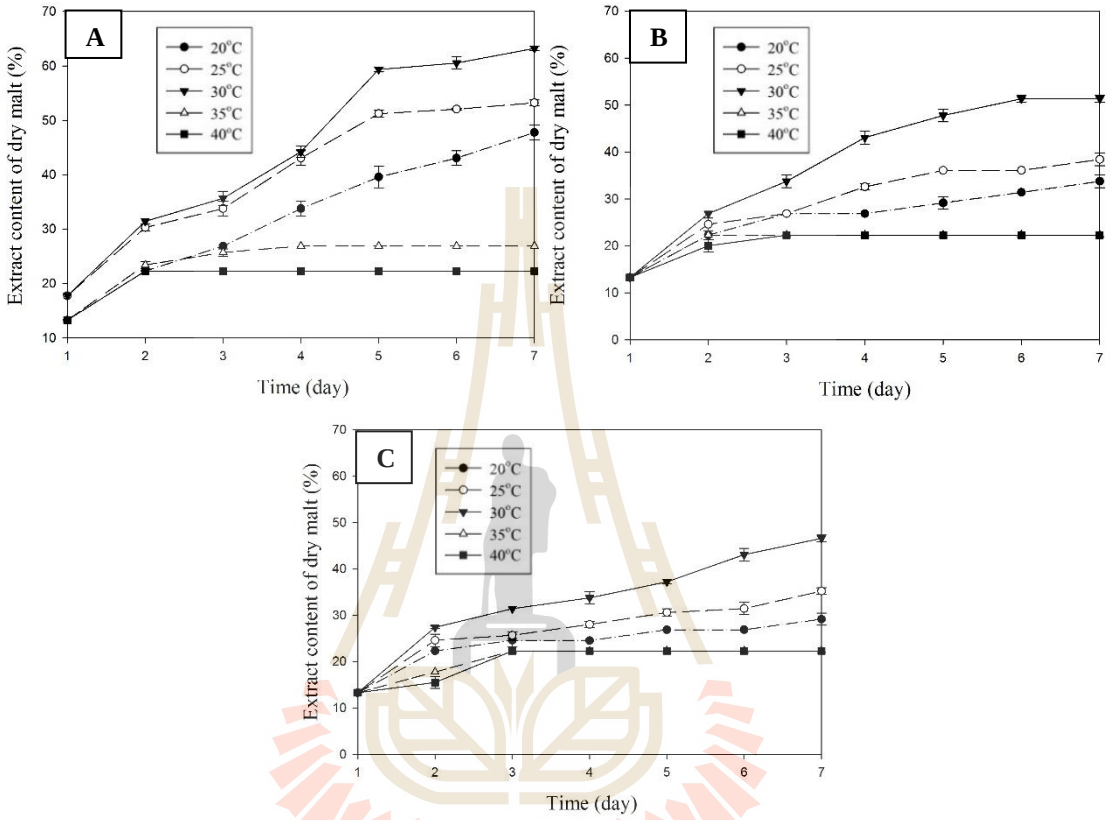

Figure 4.9 The time course of extract content in congress wort of germinated maize at the different temperature: A: steeping 24 h, B: steeping 36 h, C: steeping 48h. Steeping temperature was at 30°C.

The results revealed that steeping time, steeping temperature, germination time and germination temperature were the important factors for malt quality. The best values of enzyme activities, FAN, Kolbach index, extract content and reducing sugar was presented in maize that steeped at 30°C for 24 h, germinated at 30°C for 5 days. Therefore, these malting conditions was chosen for the next experiments.

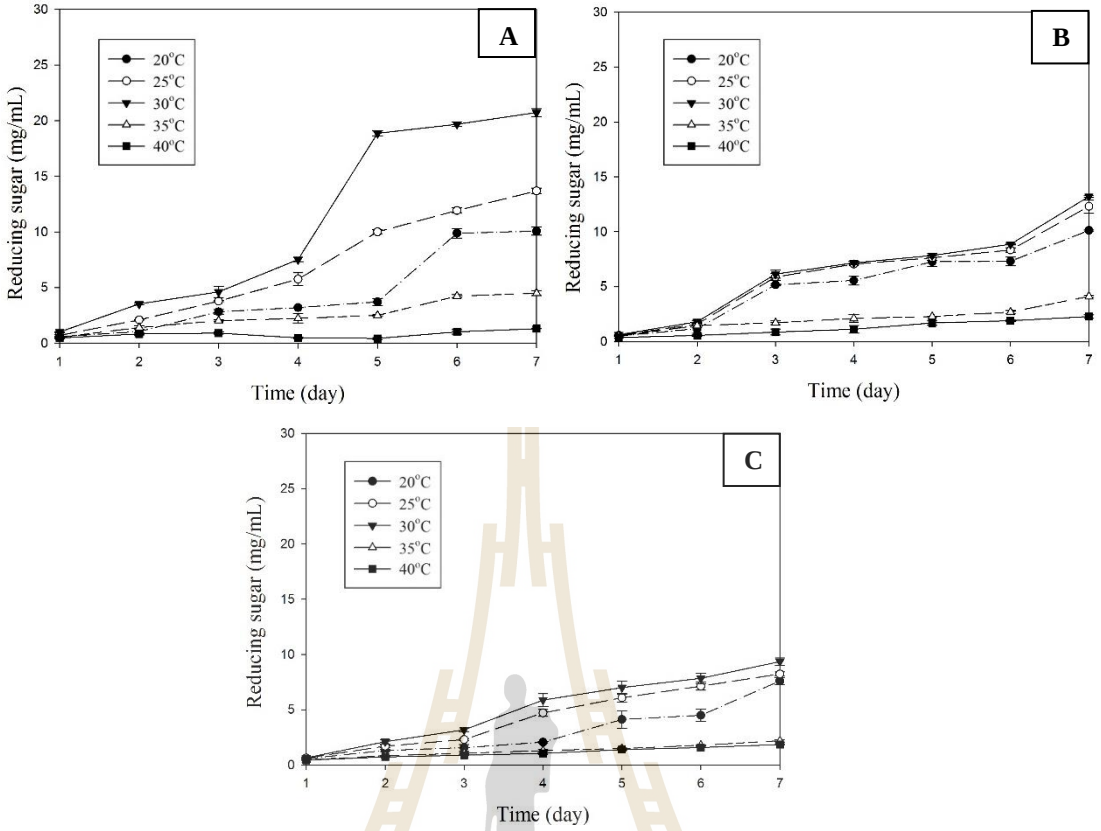

Figure 4.10 The time course of reducing sugar in congress wort of germinated maize at the different temperature: A: steeping 24 h, B: steeping 36 h, C: steeping 48h. Steeping temperature was at 30°C.

4.4 Optimization of the ratio of maize malt and barley malt for new larger beer

The different mashing profiles were applied to produce wort from the various ratio of barley malt and maize malt. The infusion mashing was used for 100% barley malt wort and decoction mashing was conducted for supplemented maize malt wort. The qualities of wort including FAN, extract content and fermentable sugar in wort were determined.

4.4.1 The effect of mashing on FAN content in the wort

The wort samples were collected every mashing steps. For 100% barley and maize malt, the worts were gathered after protein rest, α- and β-amylases, excepted for 100% maize, the sample was collected after boiling. For supplemented maize malt wort, the worts were saved after barley wort decocted with maize wort at 45^{o}C, then samples after saccharification were collect at 62^{o}C and 72^{o}C.

Table 4.5 illustrated the changing of FAN in wort in numerous ratio of the barley and maize malts. The FAN was mainly formed during enzymatic degradation of proteins at first step of mashing. After that, the FAN slightly increased and stabled until the end of mashing process. The FAN in maize malt (348.99 mg/mL) higher than barley malt (290.33 mg/mL). Therefore, when increasing the proportion of maize malt, the FAN was increased. When compared the FAN content in maize malt with another malt, the maize malt showed a worthly value. Igyor and colleagues (2001) studied the effect of malting temperature and mashing methods on sorghum wort composition. The peak of FAN in wort from sorghum malt was 177 mg/mL. Moreover, in 2010, Phiarais et al. reported the beer brewed from 100% buckwheat malt, the result showed the maximum amount of wort was 210 mg/mL. The level of FAN in final worts of each ratio in the range of 290 to 350 mg/mL, which indicated that was suitable for beer fermentation.

Table 4.5 FAN content during mashing at different ratio of barley malt and maize malt. The different superscript letter between the rows was a significant difference at $p \leq 0.05$.

Ratio (Maize:Barley)	After 45°C (mg/mL)	After 62°C (mg/mL)	After 72°C (mg/mL)	Final wort (mg/mL)
0 : 100 *	287.83±2.57^{a}	289.56±0.39^{a}	290.33±0.43^{a}	290.33±0.43
10 : 90	288.00±2.31^{a}	289.36±0.99^{a}	290.40±1.89^{a}	290.40±1.89
20 : 80	300.94±0.75^{b}	302.78±1.32^{b}	302.98±2.78^{b}	302.98±2.78
30 : 70	304.70±1.01^{b}	306.78±0.87^{b}	308.12±0.78^{b}	308.12±0.78
40 : 60	314.87±0.92^{c}	315.57±2.31^{c}	316.51±0.69^{c}	316.51±0.69
50 : 50	317.99±2.52^{cd}	318.24±2.69^{c}	319.02±1.23^{c}	319.02±1.23
60 : 40	319.91±2.12^{cd}	320.75±0.80^{c}	322.16±0.36^{cd}	322.16±0.36
70 : 30	321.32±0.81^{d}	322.41±1.45^{c}	325.94±0.92^{d}	325.94±0.92
80 : 20	332.20±2.77^{e}	338.47±2.10^{d}	341.36±2.21^{e}	341.36±2.21
90 : 10	341.68±0.67^{f}	344.58±0.98^{de}	344.92±0.68^{ef}	344.92±0.68
100 : 0 *	345.88±1.75^{f}	348.35±1.00^{e}	348.46±0.41^{f}	348.99±0.41

(*) The wort samples were collected every mashing steps in the infusion mashing process. The remaining wort samples were collected in the second mashing process of decoction mashing.

4.4.2 The effect of mashing on extract content in the wort

In the same way, collecting sample as FAN determination, the extract content of wort was analyzed from the different ratio of malt. It can be seen in Table 4.6, the soluble sugar increased along the mashing time considering the amylolytic enzyme activity. For all barley and maize malts, when starting mashing, after proteolytic rest, the extract content moderately risen, after that, it went up to maximum value after saccharification time. In case of 100% maize malt, the extract content of wort was 50.08% after it reached α-amylase rest (72ºC). However, the gelatinization temperature of maize malt was 75.6ºC and higher than the rest point. Therefore, the highest amount of the extract content was obtained after boiling point. On the other hand, when compared with supplemented wort for the decoction of mashing, the maize wort was completely extracted before mashing with barley wort, hence the extract content of these worts after protein rest were higher than wort from 100% of barley and maize malt. The final extract amount of each proportion was similar at 14.74ºP or approximate 87.33% extract.

4.4.3 The fermentable sugars in wort

Total 10 worts in various ratio of maize malt and barley malt were analyzed by using HPLC to determine fermentable sugars. Fermentable sugars of wort in each treatment including glucose, fructose, maltose, and maltotriose were shown in Table 4.7. The wort contained high concentration of maltose and maltotriose which were formed during malting stage. In addition, the glucose and sucrose components also presented in worts. The maltose in 100% maize malt wort slightly higher in wort than 100% barley

malt. In contrast, the maltotriose component in maize malt wort lower than barley malt wort.

Table 4.6 Extract content (%) of wort during mashing at different ratio of barley malt and maize malt. The different superscript letter between the rows was significant difference at $p \leq 0.05$.

Ratio (Maize:Barley)	After 45ºC (%)	After 62ºC (%)	After 72ºC (%)	Final wort (%)
0 : 100 *	32.07±0.03^{k}	74.55±0.01^{b}	87.33	87.33
10 : 90	41.31±0.01^{a}	62.18±0.02^{a}	87.33	87.33
20 : 80	44.22±0.01^{b}	74.55±0.02^{b}	87.33	87.33
30 : 70	53.09±0.01^{c}	72.06±0.02^{c}	87.33	87.33
40 : 60	56.06±0.01^{d}	76.18±0.02^{d}	87.33	87.33
50 : 50	62.18±0.01^{e}	78.52±0.02^{e}	87.33	87.33
60 : 40	65.19±0.01^{f}	71.44±0.01^{f}	87.33	87.33
70 : 30	67.18±0.02^{g}	73.23±0.02^{g}	87.33	87.33
80 : 20	74.55±0.04^{h}	78.86±0.02^{h}	87.33	87.33
90 : 10	77.69±0.01^{i}	84.56±0.02^{i}	87.33	87.33
100 : 0 *	27.03±0.01^{j}	32.70±0.04^{j}	50.08±0.07^{a}	87.33

(*) The wort samples were collected every mashing steps in the infusion mashing process. The remaining wort samples were collected in the second mashing process of decoction mashing.

The wort composition has been influenced by the ratio of maize and barley malt. In general, the total fermentable sugars in all ratio was high when compared with another experiment. Marconi et al. (2017) brewed beer from rice malt wort, the results showed lower total sugar profile at different mashing process when compared with

barley malt wort and maize malt wort.

Table 4.7 Fermentable sugars of experimental wort. The different superscript letter between the rows was a significant difference at $p \leq 0.05$.

Ratio (Maize:Barley)	Fermentable sugar (g/L)				Total (g/L)
	Maltotriose	Maltose	Glucose	Fructose	
0 : 100 *	41.90±0.61	64.32±1.12	26.36±0.63	2.23±0.09^{c}	132.81
10 : 90	41.87±0.32	65.62±0.62	26.54±0.31	2.38±0.10^{c}	136.41
20 : 80	40.96±0.18	63.96±1.31	26.97±0.16	1.56±0.06^{bc}	133.45
30 : 70	39.12±0.07	65.18±0.25	27.12±0.42	0.78±0.08^{ab}	132.20
40 : 60	41.04±0.62	67.50±0.36	26.14±0.19	1.95±0.13^{bc}	136.63
50 : 50	40.32±0.24	68.43±0.42	27.62±1.03	0.16±0.02^{a}	136.53
60 : 40	40.18±0.79	66.70±0.26	26.67±0.38	1.23±0.36^{abc}	134.78
70 : 30	38.87±1.41	67.27±2.31	27.13±0.17	1.41±0.51^{abc}	134.68
80 : 20	39.62±0.36	69.69±1.39	28.41±1.01	1.68±0.25^{bc}	139.40
90 : 10	40.81±0.65	67.12±1.61	28.31±0.51	1.89±0.43^{bc}	138.13
100 : 0 *	39.74±0.78	68.32±1.63	29.54±0.95	2.00±0.17^{b}	139.60

(*) The wort samples were collected every mashing steps in the infusion mashing process. The remaining wort samples were collected in the second mashing process of decoction mashing.

Based on the obtained results in wort parameters. The worts produced from both barley and maize malt was probably suitable for fermentation. For beer fermentation, the worts were conducted from 100% maize malt and 50:50 ratio of barley malt and maize malt was chosen. Finally, the beer from 100% barley malt was fermented as a reference.

4.5 Beer fermentation

Three kinds of wort were carried out under bottom fermentation with Saflager S-189 yeast. The fermentation was conducted until the extract was not changed within 48 h. The time course of viable cell count, FAN, reducing sugar, pH and ethanol production as well as fermentable sugars consumed (glucose, fructose, maltose and maltotriose) during fermentation were determined.

4.5.1 Beer fermentation of 100% barley malt

In the first place, the viable cells rapidly rose within first 36 h and moderately declined until the end of fermentation. In this time, the yeast used the organic compounds in the wort to increase the biomass. Therefore, the fermentable sugars in fermented wort were sharply decreased and gradually lowered at the same time. Simultaneously, the concentration of ethanol considerably went up to 96 h and was stability till the fermentation finished.

The FAN, extract content (^{o}P) and reducing sugar as the function of time were shown in Figure 4.11. These parameters were rapidly consumed from 0 to 72 h and they were kept constant afterwards. The amount of FAN was decreased dramatically during 48 h from 286.32 mg/mL to 127.83 mg/mL, after that FAN was remained stable at the equilibrium value around 93.26 ÷ 94.33 mg/mL. Chang et al. (2011) reported that the rapid FAN uptake was recorded for all fermentation modes after 24 h when they fermented beer from sorghum in FAN supplementation under very-high-gravity conditions. From the obtained result, the extract content and reducing sugar also went

down together with the rising of time. The explanation for this tendency is the utilization of yeast along fermentation.

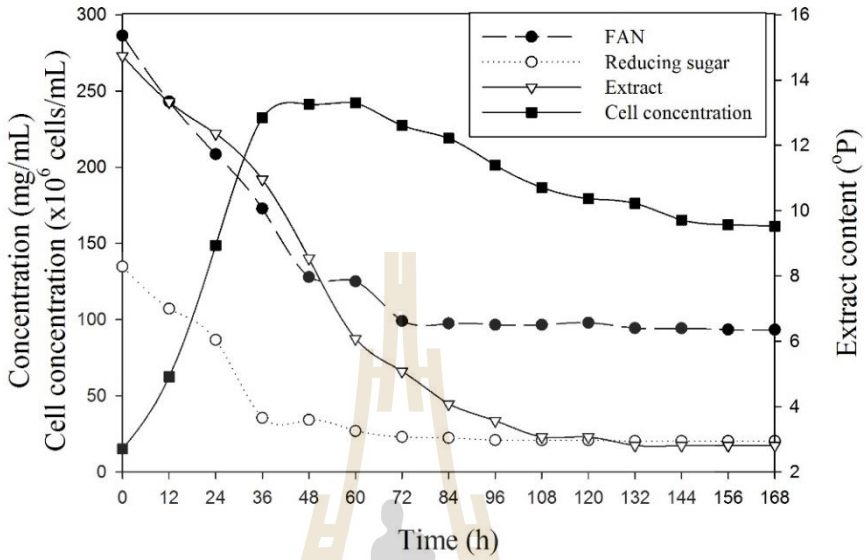

Figure 4.11 The time-course of FAN, reducing sugar, extract content and viable cell count during fermentation of 100% barley malt.

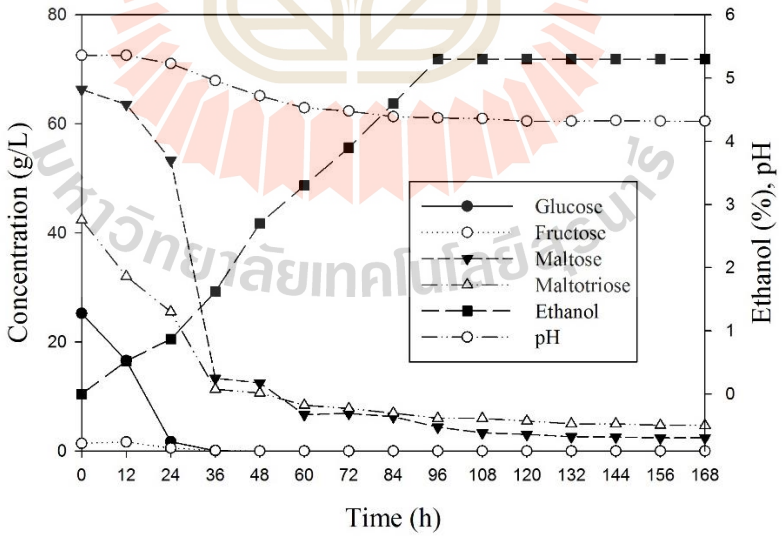

Figure 4.12 The time-course of fermentable sugars, ethanol and pH during fermentation of 100% barley malt.

The changing of pH during fermentation was caused of the oxidation of substances under anaerobic conditions. First of all, yeast utilized sugars in wort and converted into ethanol and carbon dioxide. In the meanwhile, CO_2 was released into the air in the form of gas bubbles. Practically; CO_2 was also dissolved in the aqueous solution. Then, the dissolved CO_2 produced carbonic acid in the small amount. Carbonic acid is a weak acid which will slightly reduce the pH. Thus, the pH slowly decreased during fermentation. In case of 100% barley malt beer, the pH in the beginning was 5.36 and lowered to 4.32 when finished fermentation. This result agreed with Coote and Kirsop (1976) when they investigated the factors responsible for the decrease in pH during beer fermentation.

4.5.2 Beer fermentation of 100% and supplemented maize malt

The fermentation of 100% and supplemented maize malt took longer time than 100% barley beer due to the different in fermentation conditions: mixing condition, oxygen uptake rate, fermentation tank.

As explained earlier, the consumption of FAN, extract and reducing sugar was similar to barley beer. These values decreased along the time. However, two kinds of maize beer needed more time. The FAN concentration was significantly declined in 192 h and was stayed stable later.

In addition, the fermentable sugars were utilized and decreased during fermentation. Generally, the glucose and fructose were considerably fallen and exhausted for 96 h of fermentation period for two worts. The reason is that two of these sugars were monosaccharide and were consumed before maltose and maltotriose.

Maltose and maltotriose were slightly reduced in the beginning of fermentation at 48 h. Afterwards, the significant utilization of these disaccharide and trisaccharide were occurred 48 – 216 h. In the same way, the extract yield and reducing sugar also dropped at the same time.

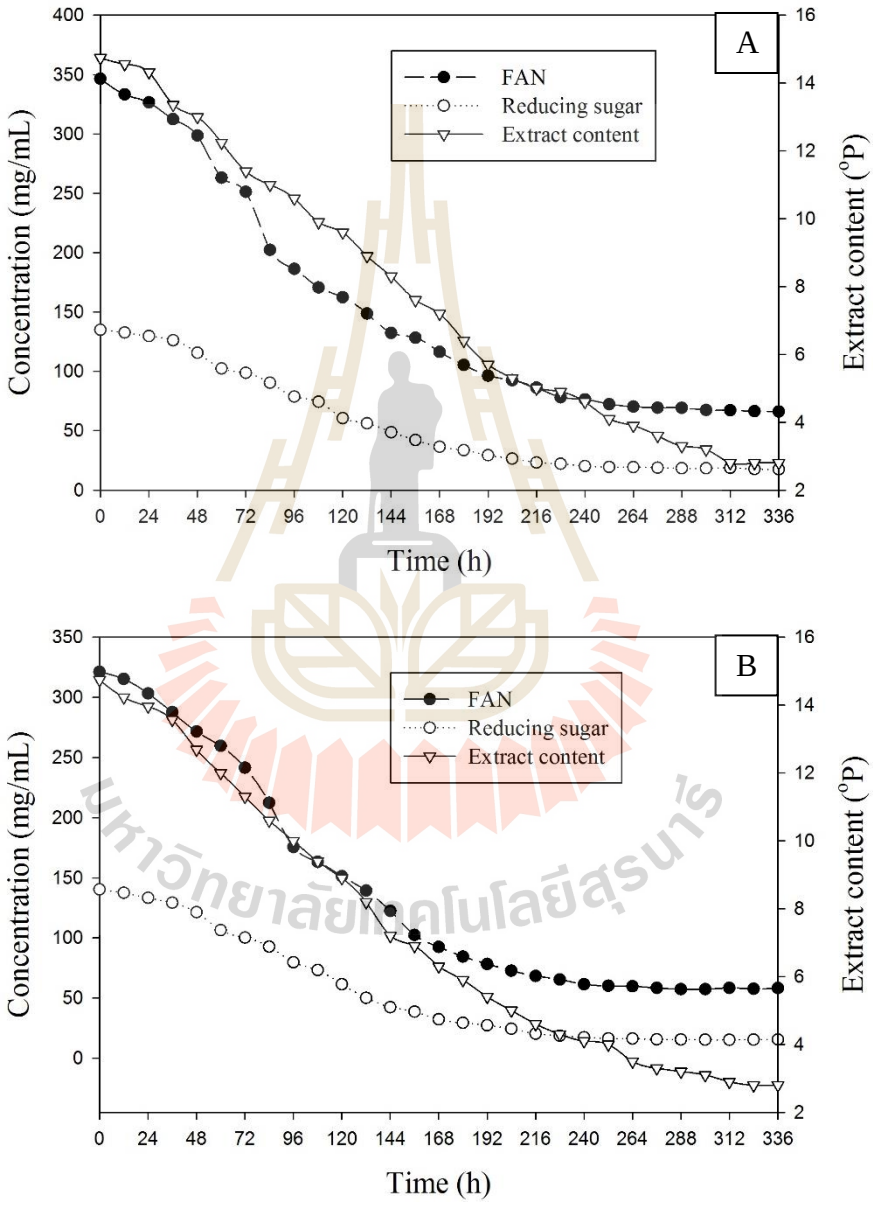

Figure 4.13 The time-course of FAN, reducing sugar and extract content during fermentation of 100% maize malt (A) and 50% supplemented maize malt (B).

As illustrated in Figure 4.13 and Figure 4.14, the analyzed ethanol was increased whereas the fermentable sugars decreased. Furthermore, the pH value went down as well as ethanol accumulation. These results were reasonably corresponded with the changing trends that were reported in the experiments of Mohammadi et al. (2011).

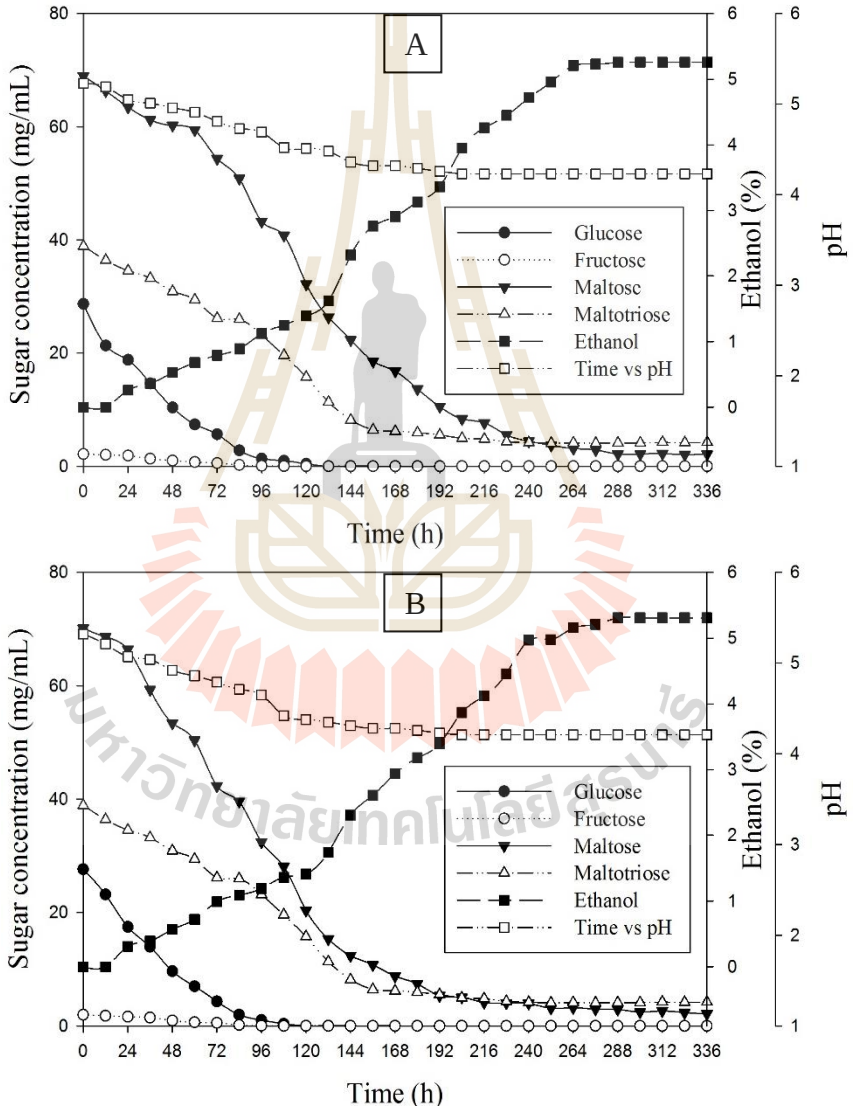

Figure 4.14 The time-course of fermentable sugars, ethanol and pH during fermentation of 100% maize malt (A) and 50% supplemented maize malt (B).

4.5.3. Chemical compounds of final beer

Flavours and aromas in beer are very important quality of the beer. The chemical compositions are obviously responsible for that properties. Volatile compounds are essential characters contributing to beer odour. They released during beer manufacture. The biosynthesis of some beer flavour was summarized in Figure 4.15. The volatile compounds in beer consists of higher alcohol or fusel alcohol, ester compounds, and free fatty acid were analyzed as summarized in Table 4.8.

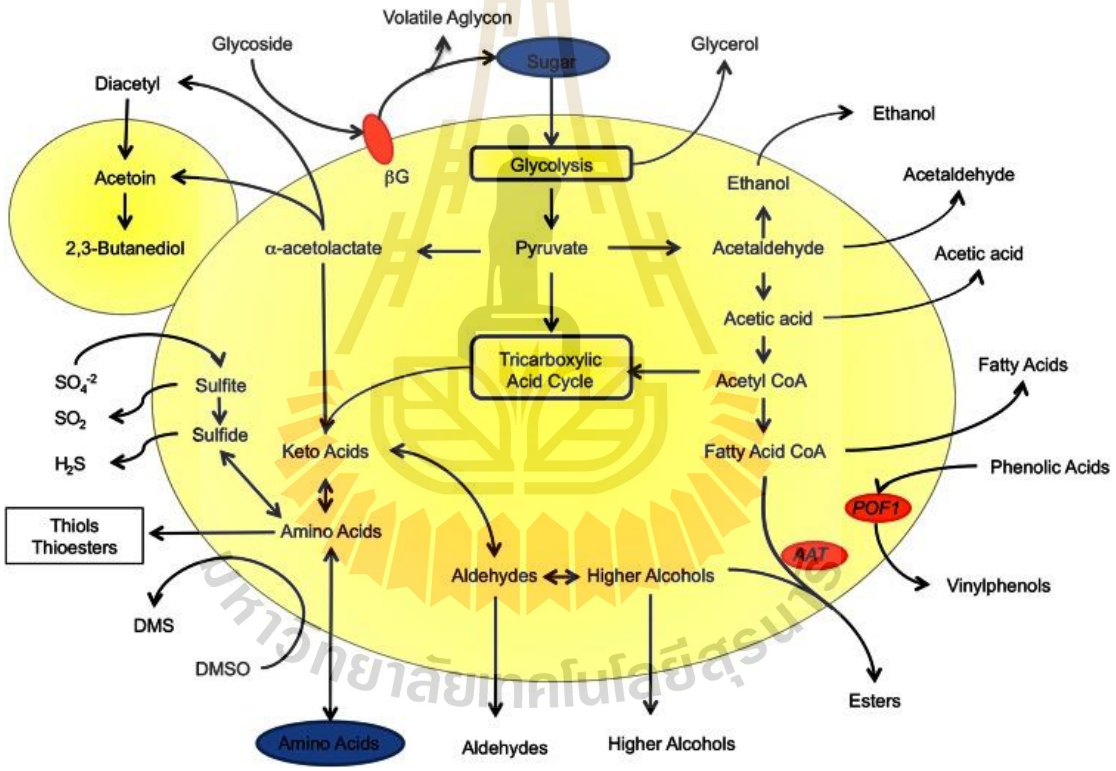

Figure 4.15 Overview of *Saccharomyces* metabolic activities influencing beer quality. This simplified schematic summarizes the main metabolic pathways linked to beer flavour modulation by *Saccharomyces*. β-glucan, β-glycosidase; DMS, dimethyl sulfide; DMSO, dimethyl sulfoxide.

Source: Bokulich and Bamforth (2013)

Isoamyl alcohol is the most quantitatively important compound for flavour in the higher-alcohol group. It influences drinkability because the beer flavour becomes heavier if isoamyl alcohol concentration increases (Olaniran et al., 2017). Isoamyl alcohol was in the range 45 – 60 ppm, which was lower with standard beer (60 to 90 ppm). Kobayashi et al. (2006) demonstrated that isobutyl alcohol has an undesirable effect on the quality of beer if its concentration surpasses 20% of the total amount of *n*-propanol, isobutyl alcohol and isoamyl alcohol.

Table 4.8 Volatile compounds in final beers. The different superscript letter between the columns was a significant difference at $p \leq 0.05$.

Compound	**Concentration (ppm)**		
	100% barley	**100% maize**	**50% maize malt**
Isoamyl acetate	57.37 ± 0.43^{a}	45.78 ± 0.12^{b}	49.07 ± 0.13^{c}
Isoamyl alcohol	58.77 ± 0.36^{a}	46.30 ± 0.08^{b}	44.36 ± 0.41^{c}
Ethyl acetate	34.27 ± 0.07^{a}	12.49 ± 0.22^{b}	18.07 ± 0.16^{c}
Ethyl caproate	17.57 ± 0.14^{a}	25.83 ± 0.16^{b}	24.37 ± 0.21^{c}
Ethyl caprate	34.75 ± 0.24^{a}	22.12 ± 0.56^{b}	25.93 ± 0.14^{c}
Ethyl caprylate	27.94 ± 0.32^{a}	31.93 ± 0.03^{b}	30.80 ± 0.52^{b}
2-phenylethyl acetate	3.11 ± 0.11^{a}	2.39 ± 0.06^{b}	3.16 ± 0.14^{a}

Esters are secondary products produced during the anaerobic metabolism of sugars by brewing yeasts and the most important compounds influencing beer flavour. Ethyl acetate, isoamyl acetate ethyl caproate, ethyl caprylate, ethyl caprate and 2-phenylethyl acetate are represented of the ester group in brewing. The ester content depends on the beer type and the original wort gravity. In bottom fermentation beers

contain ester up to 60 mg/L (Kunze, 2004). In this study, all ester compounds were in the range of standard beer. Ethyl caproate concentrations in beers from 17 to 25 mg/L. According to Šmogrovičová and Dömény (1999), the present of 5 mg/l for hexanoic acid (ethyl caproate) were characterized the cheesy, goaty, and sweaty flavour.

As shown in Table 4.7, the final beers did not contain aldehyde, diacetyl and sulfur compounds which are very high off-flavoured. This results indicated that studied beers did not contain off-flavours.

4.5.4 Sensory evaluation of final beer

The sensory analysis test was conducted by 16 assessors at Suranaree University of Technology. The process of beer evaluation consisted of five attributes including appearance, aroma, flavour, mouth-feel and overall impression. Three kinds of experimental beer and a commercial beer were used for this test. The appearance of the beer was evaluated according to the clarity, colour, bubbles or head formation. In beer made from 100% maize malt, the average score of appearance was 2.75 (normal to like). When the ratio of barley malt increased the score of appearance increased as well. The highest score presented in 100% barley malt beer was 3.98 (very like). Moreover, the color of beer in a range of 14 - 20 EBC unit. The barley beer had reddish-gold, whilst the maize beer displayed brown and the 50% maize beer presented deep amber. When increasing the amount of maize malt, the colour of the beer was darker due to high temperature mashing process which caused caramelization reaction. The beers had regular foam stability and clarity, and the judges scored from 2.75 to 3.98. Following 7

to 9 minutes there was a thin head still appeared that kept going until the end (Table 4.9).

Table 4.9 Beer foam stability and colour of final beers.

Properties	100% Barley	100% Maize malt	50% Maize malt
Collapse 10 mm (s)	105	98	102
Collapse 20 mm (s)	180	160	169
Collapse 30 mm (s)	241	228	232
Collapse 50% (s)	340	281	315
Collapse 100% (s)	516	446	469
Colour (EBC)	14	19.9	16.8

The maize beer had stronger mead's aroma than barley beer, which led to the stronger honey aroma. The aroma from two treatment 50% and 100% maize malt was non-significantly different with scored 2.38 and 2.25, respectively, which these beers were acceptable. The Pilsner beer and commercial beer were more favourable which high score from 3.4 to 3.9 (like to very like). The high ratio of barley malt the high score of favourable. After evaluating the beer's appearance and aroma, the assessors tasted the beer. Most of the examiner like flavour of beers contained barley malt ingredient. The maize beer was judged at the normal score. The final sensory component was mouth-feel when beer was evaluated. Mouth-feel is a combination of viscosity, carbonation, astringency and tactile sensation (the light or heavy on the tongue). Protein, unfermentable sugars (dextrins), beta-glucans, carbon dioxide

influenced beer's body. According to the result, the assessors liked beer made from barley, when maize beer was evaluated as acceptable.

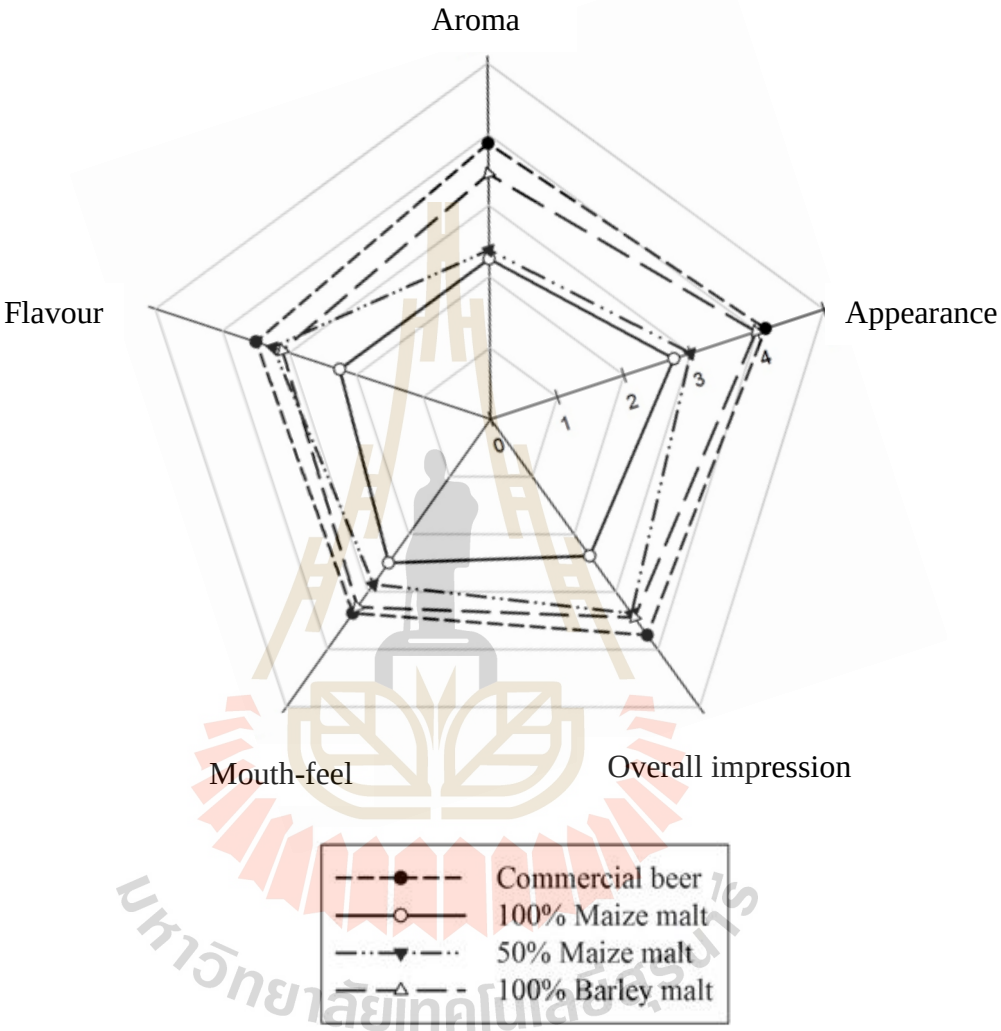

Figure 4.16 The sensory evaluation of beer tasting.

The overall impression is the description how favourite of beer. The maize beer was judged as drinkable and prefer to the next glass when two left treatments were determined as good to very good. In conclusion, all experimental beers were acceptable by assessors. Especially, the beer made from 100% maize malt was evaluated as expected which indicated that maize malt was a potential material for new beer product.

(A) (B) (C)

Figure 4.17 Photographs of lager beer at different ratio of maize malt: (A) 100% maize malt, (B) 50% maize malt and (C) 100% barley malt.

CHAPTER V

CONCLUSION

The influences of steeping durations (24, 48 and 72 h) and temperatures (20, 25, 30, 35 and 40ºC) on α- and β-amylases and protease activities in maize (Suwan 5) were investigated. The enzyme activities of maize malt were increased with temperature, however when the temperature higher 35ºC inhibited the enzyme synthesis and the maximum amount was shown at 30ºC. In addition, long period steeping the enzyme activity of maize retarded by time. Therefore, the steeping time was reduced and the advanced air-rest regime was applied. After that, the study of steeping time, germination time and germination temperature was conducted for malt quality determination. The optimal conditions which showed the highest value of malt quality in term of α- and β-amylases, protease, FAN, Kolbach index, extract content and reducing sugar were chosen when steeping at 30ºC for 24 h and germinated 5 days at 30ºC. This malting condition presented malting losses from of 10 to 14% which was in the same range of barley malting. Moreover, the malt quality of maize was compared with commercial barley malt. The result reported that the enzyme activities, FAN of maize malt were slightly higher than barley malt, in contrast, the Kolbach index and extract content were lower due to the high gelatinization temperature of maize.

The different mashing profiles were applied to produce wort from the various ratio of barley malt and maize malt. The wort composition have been influenced by the ratio

of maize and barley malt. The FAN in maize malt (348.99 mg/mL) higher than barley malt (290.33 mg/mL). Therefore, when increasing the proportion of maize malt, the FAN was increased. The final extract amount of each proportion was approximate 14.74^{o}P. The total fermentable sugars was not significantly different between each treatment. The results of FAN, extract content and fermentable sugars in various wort were indicated that all proportion was suitable for beer fermentation.

Three kinds of wort including 100% maize malt, 100% barley malt and 50% maize malt were carried out under bottom fermentation with Saflager S-189 yeast. The fermentation was conducted until the extract was not changed within 48 h. The utilization of FAN and fermentable sugars and in the generation of ethanol were measured during fermentation. The ethanol concentration in three final beers was approximately 5.1% (v/v). The amount of maize malt was affected by the colour of beer because of Maillard and caramelization reaction of maize during mashing and boiling.

Some of volatile compounds of beer consist of higher alcohol, ester and free fatty acid were determined. Isoamyl alcohol was in the range 45 – 60 ppm, which was lower than standard beer (60 – 90 ppm). The ester compounds were in range of standard beer. The final beers did not contain aldehyde, diacetyl and sulfur compounds which are very high off-flavored.

The sensory test of maize beer was judged as drinkable and prefer to the next glass when supplemented and 100% barley beer showed good to very good properties. This research proved the ability of maize for brewing technology as a unique product accepted by the consumer.

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APPENDICES

APPENDIX A

STANDARD CURVES

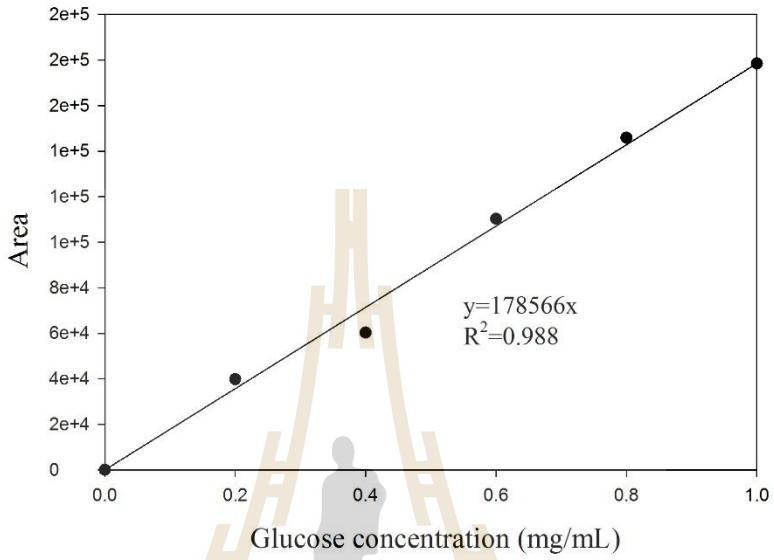

Figure 1A Standard curve of glucose using Rezex ROA-Organic Acid column with H_2SO_4 0.05 N mobile phase and RI detector.

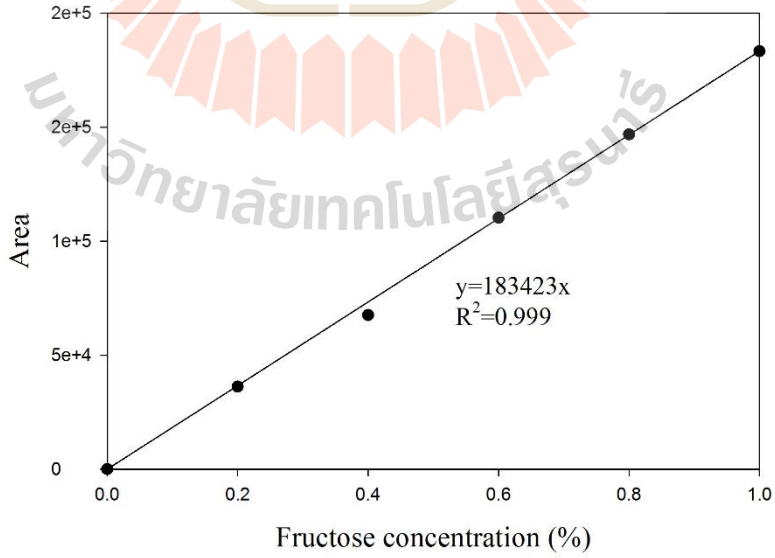

Figure 2A Standard curve of fructose using Rezex ROA-Organic Acid column with H_2SO_4 0.05 N mobile phase and RI detector.

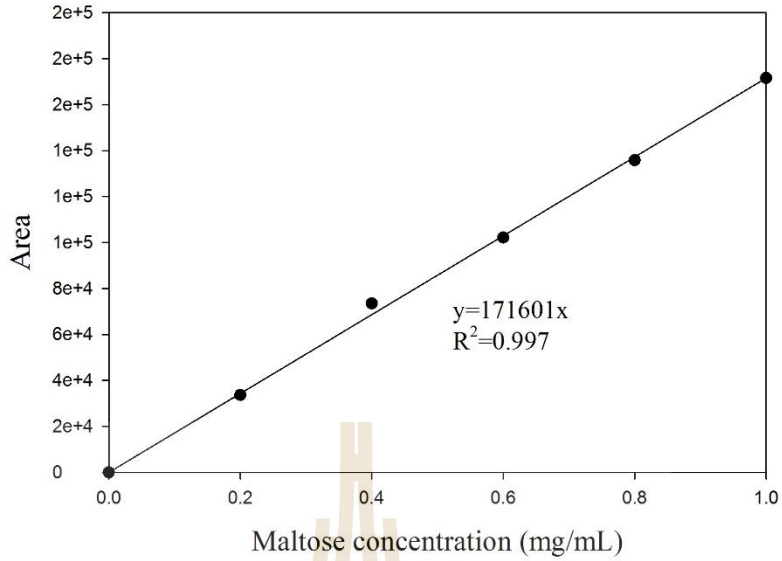

Figure 3A Standard curve of maltose using Rezex ROA-Organic Acid column with H_2SO_4 0.05 N mobile phase and RI detector.

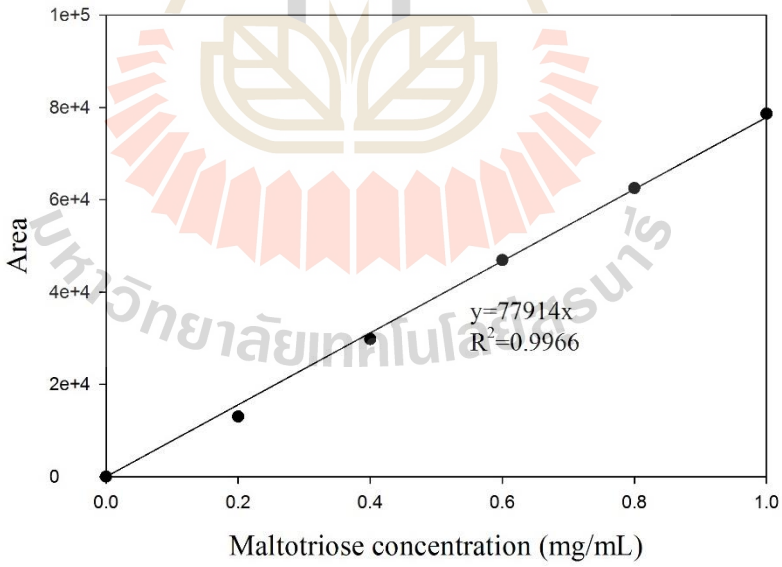

Figure 4A Standard curve of maltotriose using Rezex ROA-Organic Acid column with H_2SO_4 0.05 N mobile phase and RI detector.

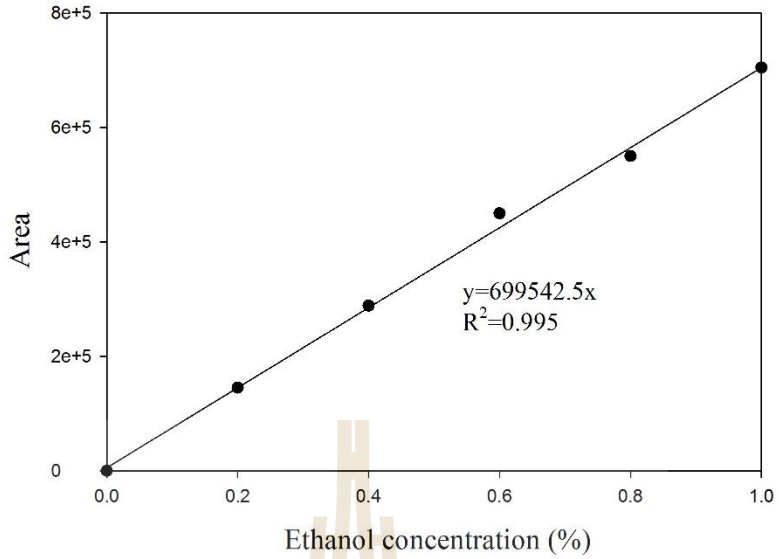

Figure 5A Standard curve of Ethanol using Rezex ROA-Organic Acid column with H_2SO_4 0.05 N mobile phase and RI detector.

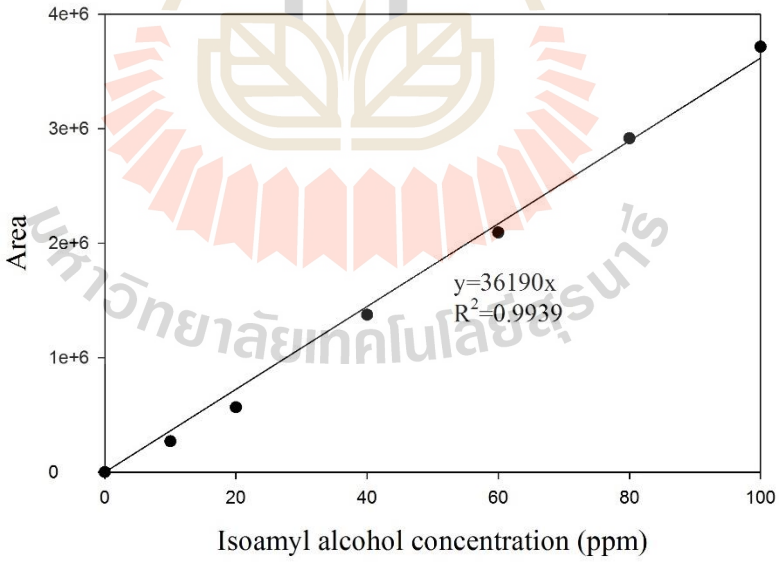

Figure 6A Standard curve of isoamyl alcohol by GC-MS using DB-WAS column with SPME technique.

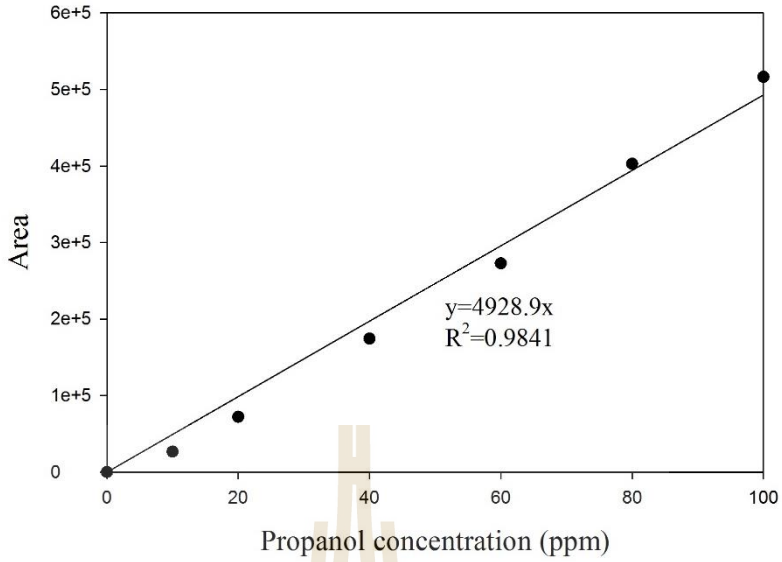

Figure 7A Standard curve of propanol by GC-MS using DB-WAS column with SPME technique.

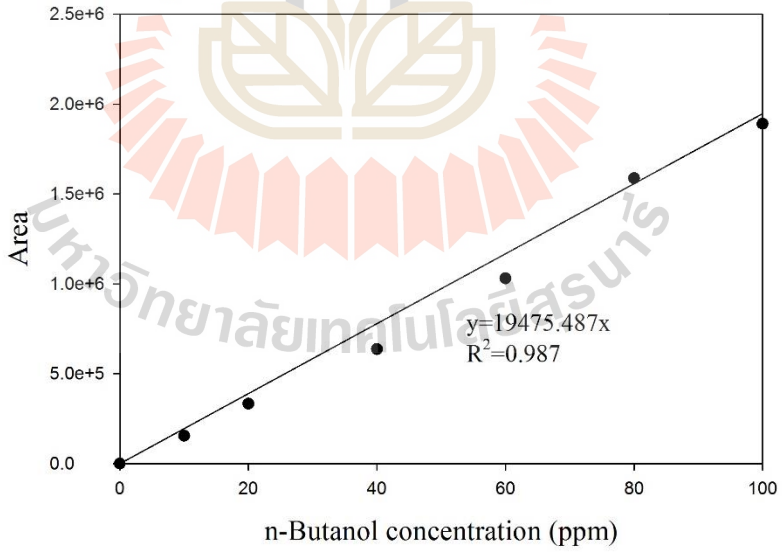

Figure 8A Standard curve of n-butanol by GC-MS using DB-WAS column with SPME technique.

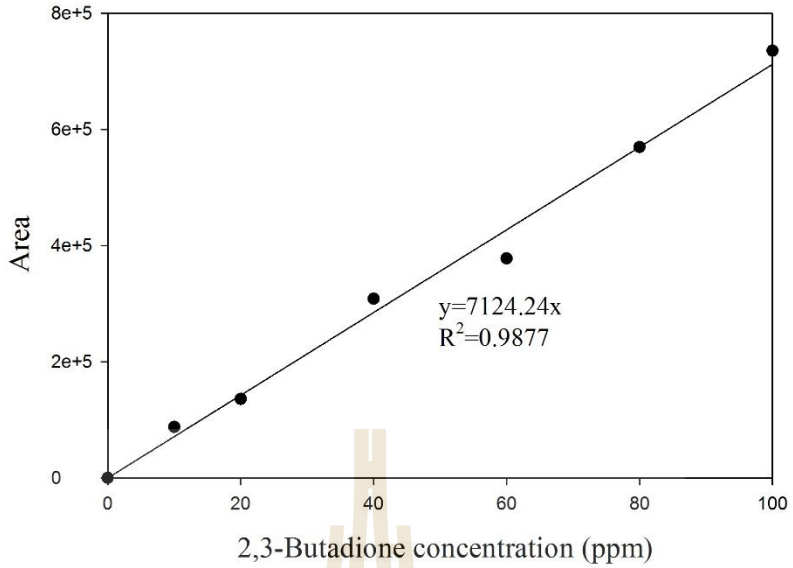

Figure 9A Standard curve of 2,3-butadione by GC-MS using DB-WAS column with SPME technique.

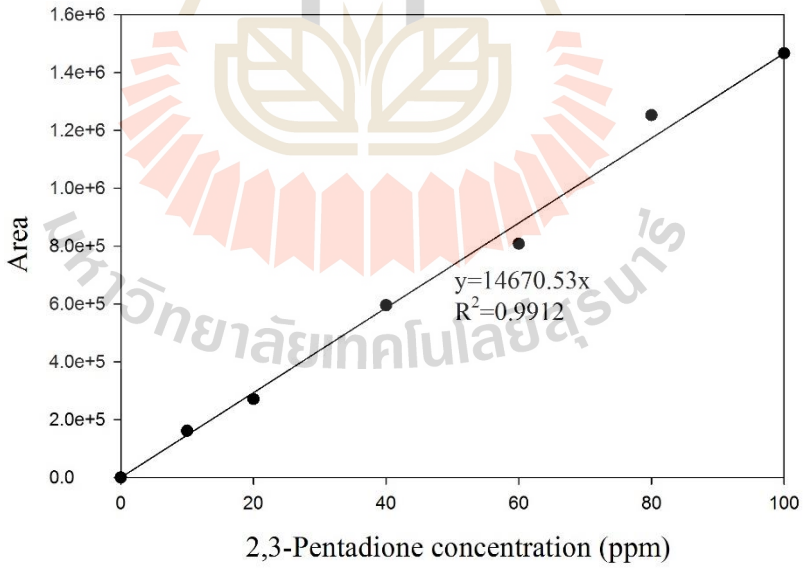

Figure 10A Standard curve of 2,3-pentadione by GC-MS using DB-WAS column with SPME technique.

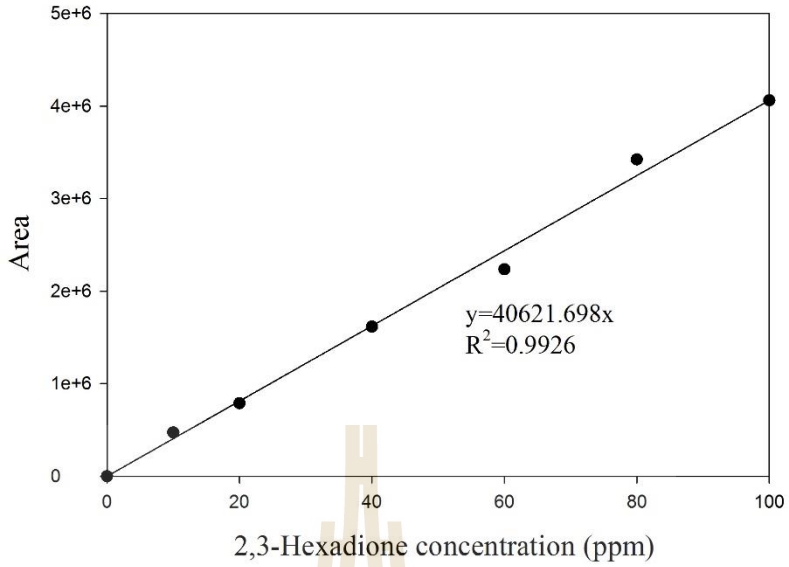

Figure 11A Standard curve of 2,3-hexadione by GC-MS using DB-WAS column with SPME technique.

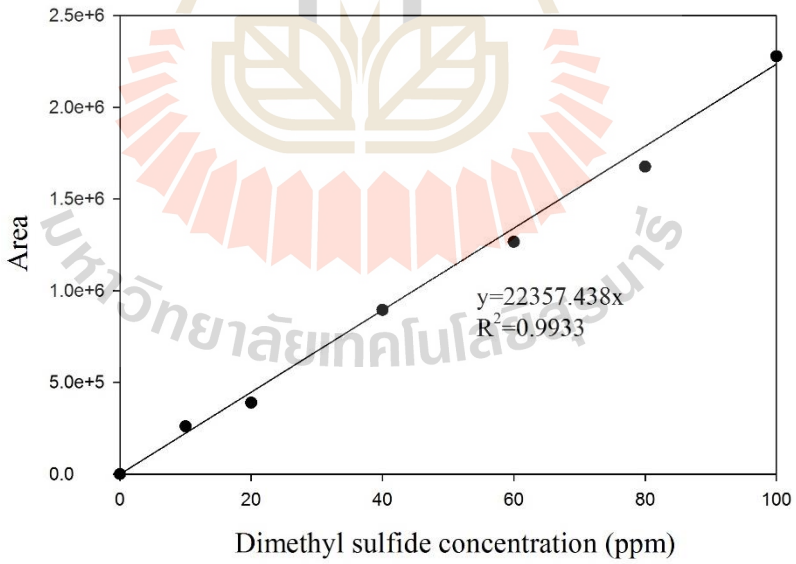

Figure 12A Standard curve of dimethyl sulfide by GC-MS using DB-WAS column with SPME technique.

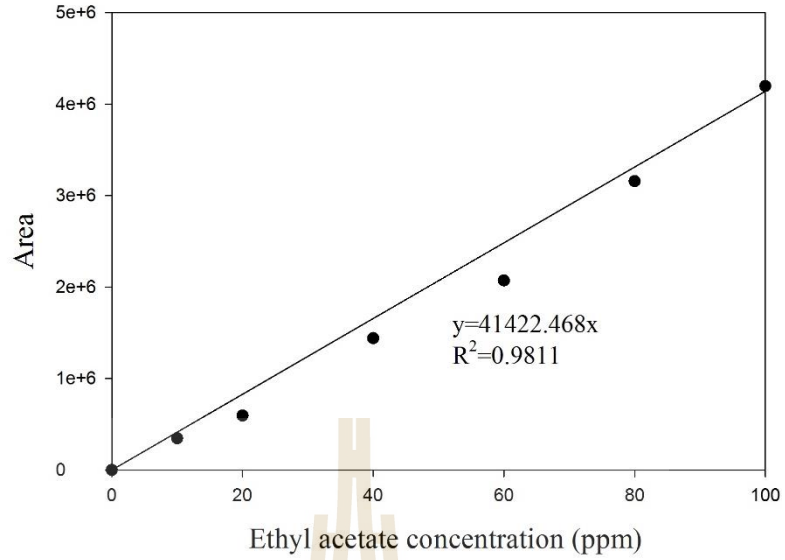

Figure 13A Standard curve of ethyl acetate by GC-MS using DB-WAS column with SPME technique.

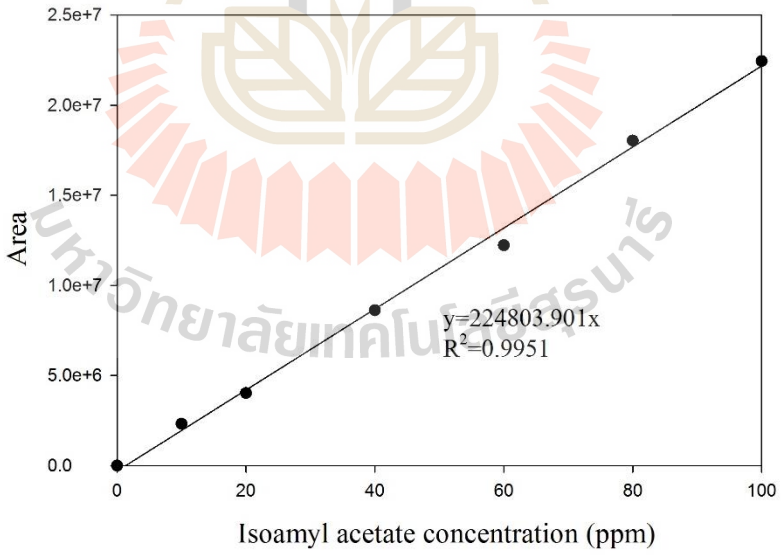

Figure 14A Standard curve of isoamyl acetate by GC-MS using DB-WAS column with SPME technique.

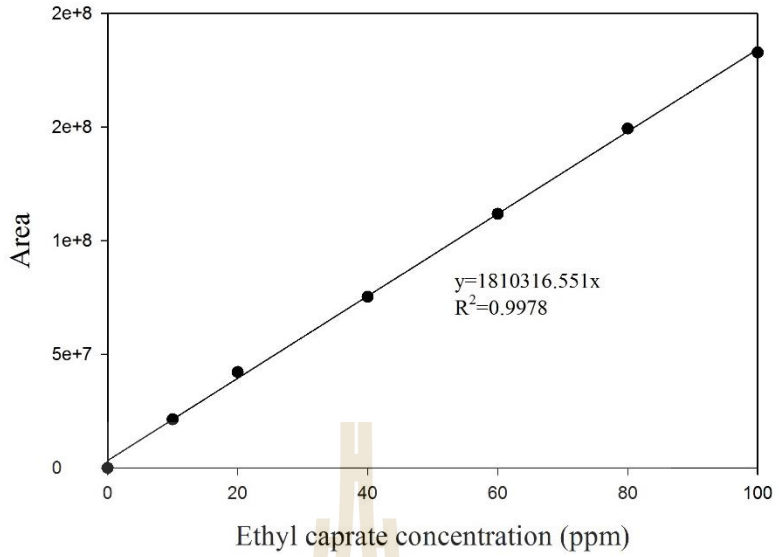

Figure 15A Standard curve of ethyl caprate by GC-MS using DB-WAS column with SPME technique.

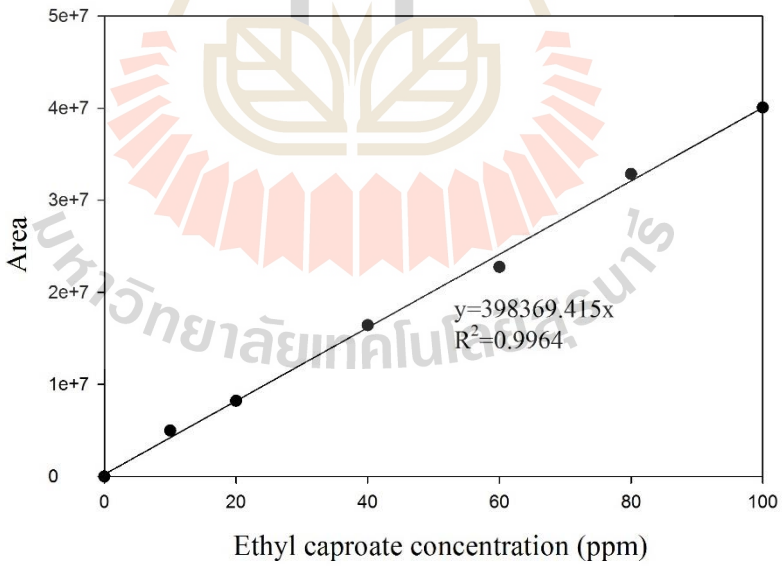

Figure 16A Standard curve of ethyl caproate by GC-MS using DB-WAS column with SPME technique.

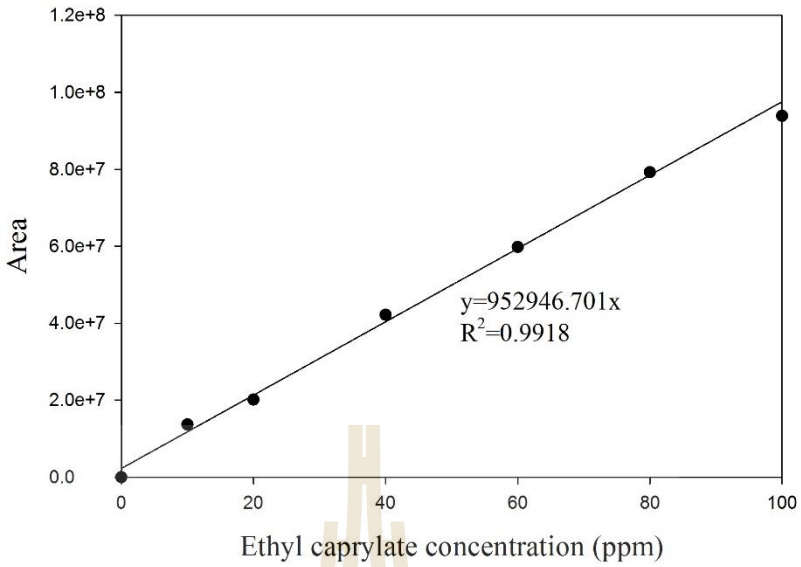

Figure 17A Standard curve of ethyl caprylate by GC-MS using DB-WAS column with SPME technique.

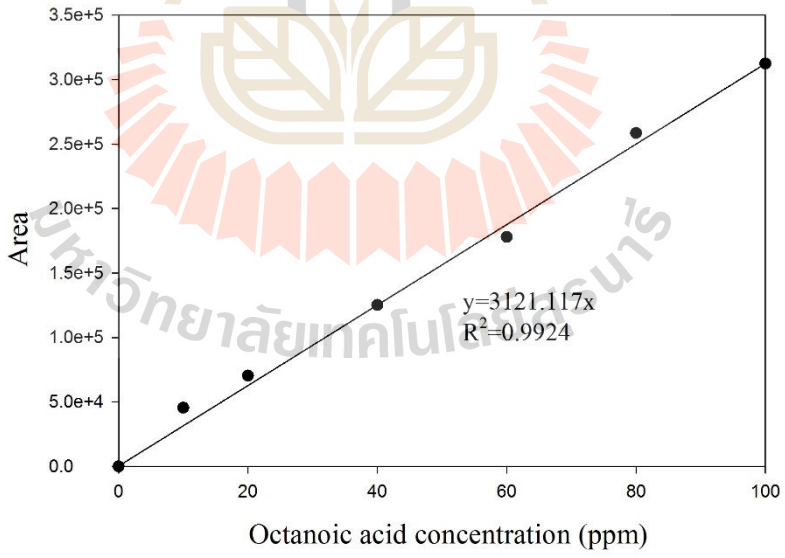

Figure 18A Standard curve of octanoic acid by GC-MS using DB-WAS column with SPME technique.

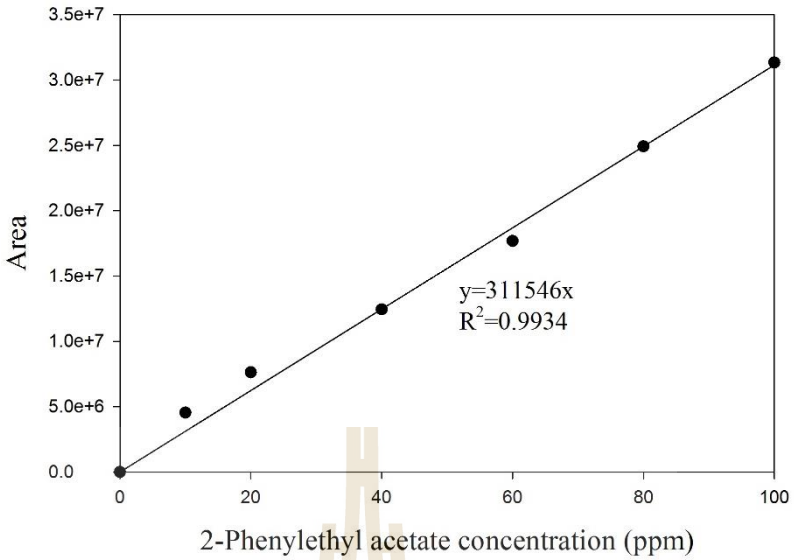

Figure 19A Standard curve of 2-Phenylethyl acetate by GC-MS using DB-WAS column with SPME technique.

APPENDIX B

CHROMATOGRAMS

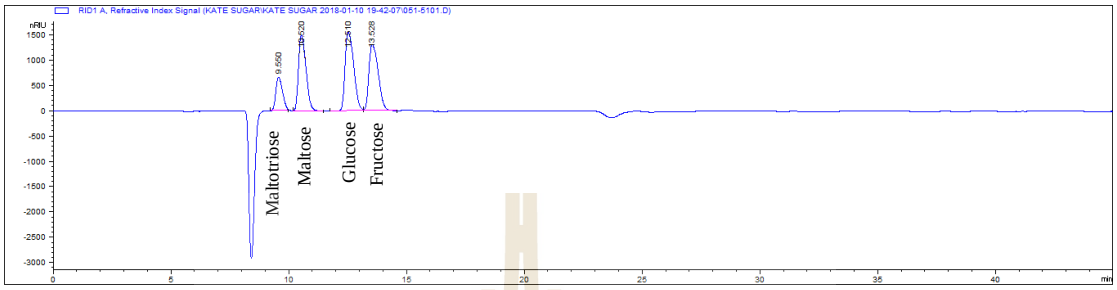

Figure 1B HPLC chromatogram of 0.2 g/L of each standards glucose, fructose, maltose and maltotriose using Rezex ROA-Organic Acid column with H_2SO_4 0.05 N mobile phase and RI detector.

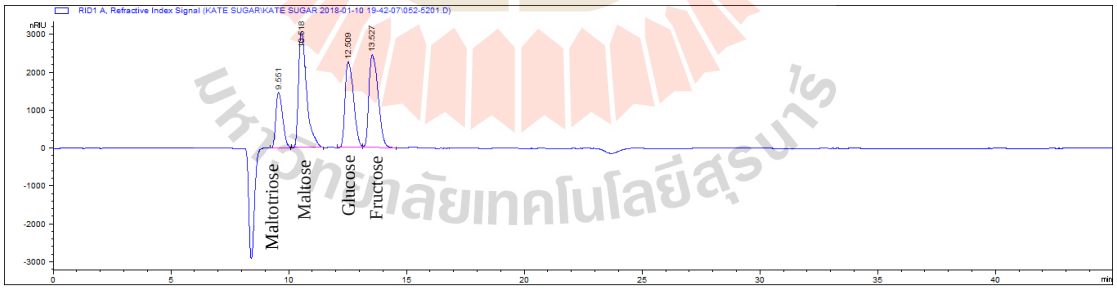

Figure 1B HPLC chromatogram of 0.4 g/L of each standards glucose, fructose, maltose and maltotriose using Rezex ROA-Organic Acid column with H_2SO_4 0.05 N mobile phase and RI detector.

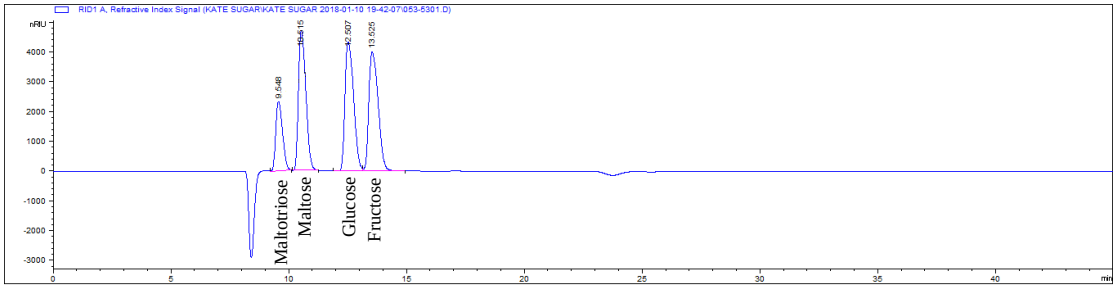

Figure 1C HPLC chromatogram of 0.6 g/L of each standards glucose, fructose, maltose and maltotriose using Rezex ROA-Organic Acid column with H_2SO_4 0.05 N mobile phase and RI detector.

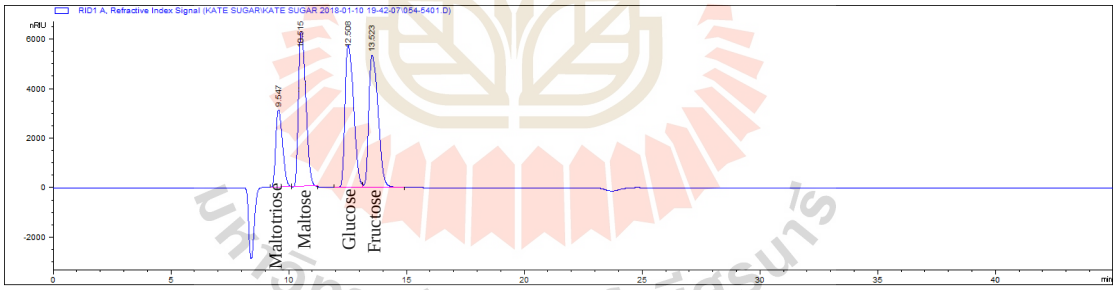

Figure 1D HPLC chromatogram of 0.8 g/L of each standards glucose, fructose, maltose and maltotriose using Rezex ROA-Organic Acid column with H_2SO_4 0.05 N mobile phase and RI detector.

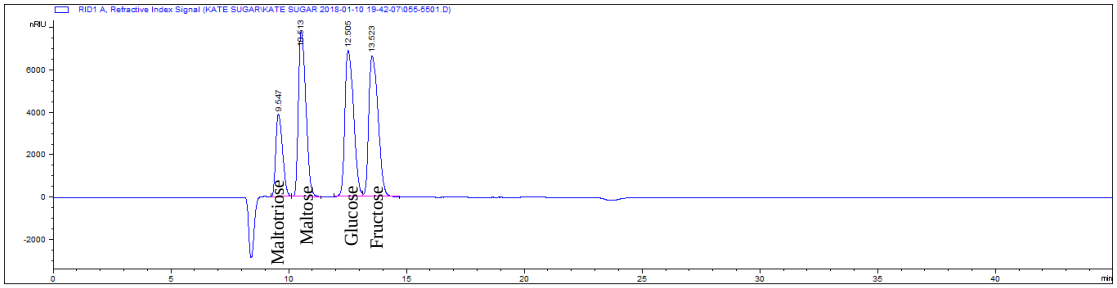

Figure 1E HPLC chromatogram of 1.0 g/L of each standards glucose, fructose, maltose and maltotriose using Rezex ROA-Organic Acid column with H_2SO_4 0.05 N mobile phase and RI detector.

BIOGRAPHY

Miss Thu Thi Anh Le was born on November 28, 1992 in Thua Thien Hue province, Vietnam. In 2010, she studied in major Food Science and Technology, College of Agriculture and Forestry in Hue University, Thua Thien Hue, Vietnam. During that time, she was a member of "Student Exchange Program in Thailand" in Rajamangala University of Technology Isan in 2013 and 2014. She took part in "Technology transfer for food product and food product analysis" and "Art and Cultural in Mekong River Region" at 2013. In 2014, she joined in "International training on Agriculture and Thai traditional medicine". She graduated the Bachelor's Degree in Food Science and Technology in 2015. After that, she continued her Master's Degree in Biotechnology at School of Biotechnology, Institute of Agriculture Technology at Suranaree University of Technology (SUT), Nakhon Ratchasima, Thailand. During her study, she received financial support from Suranaree University of Technology. Her expertise is alcoholic beverage, consists of wine processing and beer processing. Her dissertation title was "Maize malt supplementation of barley for the new beer production".