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Effect of different mashing on non-alcoholic beer quality from Thai rice malt

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Abstract

The effect of mashing temperature on the quality of wort and beer from Thai rice malt was studied using two non-glutinous rice varieties; Homnin and PathumThani 1. The mashing program was carried out at two different temperatures, 65° and 75°C, for the sugar rest period. It was found that the chemical properties of wort from infusion mash at 65° and 75°C were not different in % extract, reducing sugar and free amino nitrogen contents. However, sugar composition from different worts was significantly different. Sugar composition of wort from infusion mash at 65°C gave 8.65 g/100g maltose and 0.98 g/100g glucose while at 75°C gave 3.26 g/100g maltose and 6.70 g/100g glucose. Wort fermentation was prepared in batch sizes of 20 litre (L) using yeast *Saccharomyces cerevisiae* RSU no.10. The beer qualities of color value, turbidity, pH, and reducing sugar were not different. It was also found that the chemical properties of both beer patterns were the same in quantities of Free Amino Nitrogen (FAN), % extract, % alcohol and bitterness. The bio-functional compounds content corresponded to specification of a healthy beer product which focused on antioxidant, phenolic compound and GABA and were not significantly different, except for the thiamine content remaining in the final product. It was found that thiamine content in beer from infusion mash at 75°C wort was higher than beer from infusion mash at 65°C wort. The sensory evaluation of beers indicated that appearance, aroma, taste, mouth-feel and overall impression of non-alcoholic beer from both beer patterns were judged as drinkable and preferred to the commercial non-alcoholic beer from Germany, namely Clausthaler. The results obtained from this research clearly indicated that non-alcoholic beer from Thai rice malt influenced perception of the consumer and could be used as alternative healthy beverage with an acceptable quality.

Keywords**:** *mashing, infusion mash, sugar composition, rice beer, non-alcoholic beer, healthy beverage*

บทคัดย่อ

การศึกษาผลของอุณหภูมิในการต้มย่อยต่อคุณภาพน้ำเวิร์ตและเบียร์จากข้าวไทยที่เตรียมจากมอลต์ข้าวเจ้าหอมนิลและปทุมธานี 1 ที่ผ่านการคัดเลือกสายพันธุ์โดยปรับความแตกต่างในโปรแกรมการต้มย่อยแบบอินฟิวส์ชั่นเพื่อให้เกิดการย่อยแป้งเป็นน้ำตาลที่สองอุณหภูมิที่แตกต่างกันที่ 65 และ 75 องศาเซลเซียส พบว่าคุณสมบัติทางเคมีของน้ำเวิร์ตที่ผ่านการต้มย่อยที่อุณหภูมิ 65 และ 75 องศาเซลเซียส แสดงปริมาณสารสกัด น้ำตาลรีดิวส์ และแอมิโนไนโตรเจนอิสระไม่แตกต่างกัน อย่างไรก็ตาม พบความแตกต่างในองค์ประกอบของชนิดน้ำตาลในน้ำเวิร์ตทั้งสองอุณหภูมิอย่างมีนัยสำคัญ โดยการย่อยแป้งเป็นน้ำตาลที่อุณหภูมิ 65 องศาเซลเซียส มีปริมาณมอลโตสร้อยละ 8.65 โดยน้ำหนัก และกลูโคสร้อยละ 0.98 โดยน้ำหนัก ในขณะที่การย่อยแป้งเป็นน้ำตาลที่อุณหภูมิ 75 องศาเซลเซียส มีปริมาณมอลโตสร้อยละ 3.26 โดยน้ำหนัก และกลูโคสร้อยละ 6.70 โดยน้ำหนัก การหมักเบียร์ที่เตรียมจากน้ำเวิร์ตอุณหภูมิ 65 และ 75 องศาเซลเซียส ดำเนินการในถังหมักขนาด 20 ลิตรโดยใช้ยีสต์ *Saccharomyces cerevisiae* RSU No.10 ไม่พบความแตกต่างในคุณภาพของเบียร์ด้านค่าสี ความขุ่น ความเป็นกรดด่าง น้ำตาลรีดิวส์ และพบว่าคุณสมบัติทางเคมีของเบียร์ที่เตรียมจากน้ำเวิร์ตทั้งสองอุณหภูมิมีปริมาณสารสกัด แอมิโนไนโตรเจนอิสระ แอลกอฮอล์และค่าความขมอยู่ในระดับเดียวกัน ผลการศึกษาปริมาณสารชีวกิจกรรมในเบียร์ที่มีผลดีต่อสุขภาพชนิดฟีนอลิกส์ กิจกรรมการต้านอนุมูลอิสระ และสารกาบา พบว่าไม่แตกต่างกันในเบียร์ที่เตรียมจากน้ำเวิร์ตทั้งสองอุณหภูมิ ในขณะที่พบว่าปริมาณวิตามินบี 1 ในเบียร์ที่เตรียมจากน้ำเวิร์ตอุณหภูมิ 75 องศาเซลเซียสมีปริมาณที่สูงกว่า สำหรับผลการทดสอบทางประสาทสัมผัสทางลักษณะปรากฏ กลิ่นหอม รสชาติ ความรู้สึกในปากและความประทับใจโดยรวมในผลิตภัณฑ์เบียร์ไร้แอลกอฮอล์พบว่าอยู่ในระดับใกล้เคียงกับเบียร์ทางการค้าของเยอรมันนียี่ห้อ Clausthaler ชี้ให้เห็นว่าผลิตภัณฑ์เบียร์ไร้แอลกอฮอล์จากข้าวไทยได้รับการยอมรับและมีศักยภาพในการพัฒนาเป็นเครื่องดื่มทางเลือกเพื่อสุขภาพ

คำสำคัญ: *การต้มย่อย, อินฟิวชันแมช, องค์ประกอบน้ำตาล, เบียร์ข้าว, เบียร์ไร้แอลกอฮอล์, เครื่องดื่มสุขภาพ*

1. Introduction

The potential of Thai rice as an important source of industrial brewing material has been long recognized (Puangwerakul, 2007). Rice is regarded as an herb as it is rich in many vitamins that have properties in preventing and curing many diseases (European Herbal Infusions Association, EHIA, 2013). It has been reported that germinated rice is highly nutritious and possesses many bio-functional compounds, such as phenolic compounds, dietary fiber, and γ-aminobutyric acid (GABA) (Komatsuzaki et al., 2007; Ohtsubo, Suzuki, Yasui, & Kasumi, 2005; Chavana, Kadamb, & Beuchat, 1989). Moreover, it has also been shown to decrease some antinutrients, such as phytic acid (Watchararpapaiboon, Laohakunjit, & Kerdchoechurn, 2010). Germinated rice products manufactured and sold commercially in the Thailand food industry are edible rice; germinated brown rice and malted rice. Germinated Thai rice in the beverage industry has rarely been found. For beer production, rice used as adjuncts, also known unmalted rice, was not the main raw material for European-type lager beer production (Stewart, 2006; Vinh, Viet, & Mai, 1993). Recently, Usansa et al. (2011) reported that two black rice varieties, black non-waxy rice and black waxy rice malts had potential for use as raw material for producing beers with an acceptable quality (Usansa et al., 2011). There are many reports of rice malt production (Usansa, Sompong, Wanapu, Boonkerd, & Teaumroong, 2009; Usansa et al., 2011; Capanzana & Buckle, 1997). Thus, the use of rice malt as a major raw material for brewing in Thai breweries is still a new challenge. Moreover, the beer market is witnessing a significant increase in the consumption of low- and non-alcoholic beer mainly due to health reasons, safety reasons in the workplace or on the roads, and strict social regulations. Also, there are countries where alcohol consumption is completely forbidden by law. Therefore, non-alcoholic beer production with satisfactory consumer acceptance has recently given rise to increased technological and economic interest (Sohrabvandi, Mousavi, Razavi, Mortazavian, & Rezaei, 2010). The consumption of non-alcoholic beer appears to enhance health benefits as has been reported (Alvarez, Correa, Navaza, & Riverol, 2000; Codoner-Franch et al., 2013). Therefore, non-alcoholic beer, especially in different beer brewing styles, has been growing in popularity around the world not only by health-conscious consumers, but also by Muslims who now account for almost a third of worldwide sales. According to German law, a beer called alkoholfrei or free of alcohol may contain up to 0.5% alcohol by weight. Non-alcoholic beer can be made in several different ways which involves interrupting the fermentation process. There are several methods that remove the alcohol from the finished beer, including vacuum distillation, reverse osmosis, and evaporation. However the process for brewing non-alcoholic beers always starts out like normal beer (German Beer Institute, 2013).

Mashing is the most important process in wort production. During mashing, the grist and water are mixed together and the contents of the malt are brought into solution. The resulting extract, obtained by the action of enzymes, is then allowed to act at optimum temperature. Only soluble substances can pass into beer. The aim of mashing is to form as much extract and as good an extract as possible (Kunze, 2010). There are many reports supporting the differences of malt required and the different types of mashing and temperature programs. Moreover, there are many reports of improving the mashing procedure to increase extract yield, wort quality and investment costs (Kongkaew, Usansa, & Wanapu, 2012; Goode, Wijngaard, & Arendt, 2005; Igyor, Ogbonna, & Palmer, 2001; Alvarez et al., 2000; MacGregor, Bazin, Macri, & Babb, 1999). There are no reports that the mashing process has been optimized to produce suitable amounts of fermentable sugars that yeast metabolize to alcohol for non-alcoholic beer making in Thailand. This work used raw material reported by Kiadbanphai, Chahom, Nalad, & Kitluekiat (2012). In their report, they found that both PathumThani 1 and Homnin varieties had suitable chemical qualities in malt and wort characteristics. The main focus of this study was designed to optimize the effect of mashing method, by using different temperatures for glucose and maltose production, on wort composition and final non-alcoholic beer quality, its sensory characteristics as well as its concentration of health-benefit compounds. Therefore, the results of this study could be used to consider non-alcoholic beer as an alternative choice for an innovative health drinks for consumers.

2. Objectives

This study aimed to evaluate the effect of mashing temperature on the quality of wort and beer from Thai rice malt varieties; Homnin and PathumThani 1.

3. Materials and methods

3.1 Raw material

Two-Thai rice malt cultivars made from sprouted paddy rice; PathumThani 1 and black rice; Homnin, were obtained from the Community Farming Cooperative Enterprise honoring His Majesty the King at Thangyao Village, Sahmkhok District, PathumThani Province. Manufacturing time: November 2012. Barley malt was kindly supplied by Cosmos Brewery (Thailand), Ltd. for use as a control. The yeast strain *Saccharomyces cerevisiae* RSU No.10 was obtained from RSU Innovation Products. Ltd.

3.2 Grist preparation

Rice malt was milled by pin mill to give fine particle sizes of 200 micron grist. The mixture ratio of PathumThani 1 grist and Homnin grist was 1: 1. Grist was mashed with distilled water at grist water ratio 1:4 by weight and operated with different wort preparation.

3.3 Wort preparation

The treatment of wort for mashing of rice malt was produced by two different mashing procedures using the mashing bath at two different temperatures of 65°C and 75°C for the sugar rest period. Standard congress wort (control) for mashing of (barley) malt was produced by the EBC method (EBC, 1987).

3.3.1 Wort from mashing at 65 °C

Rice malt grits 200 g were mixed with distilled water 800 ml for 30 min at 45°C. The mash temperature was increased to 65°C and then an additional 400 ml of distilled water at 65°C was added. The mash was maintained at the temperature of 65°C for 1 h. Finally, the temperature was increased to 79°C for 10 min and then cooled to 20°C and total weight was adjusted to 1,800 g.

3.3.2 Wort from mashing at 75 °C

Rice malt grits 200 g were mixed with distilled water 800 ml for 30 min at 45°C. The mash temperature was increased to 65°C and then an additional 400 ml of distilled water at 65°C was added and held at 65°C for 30 min. Then the temperature was increased to 75° for 30 min. Finally the temperature was increased to 79°C for 10 min and then cooled to 20°C and total weight adjusted to 1,800 g.

3.3.3 Standard congress wort

Malt grits 200 g were mixed with distilled water 800 ml for 30 min at 45°C. The mash temperature was increased 1°C per a minute for 25 min to 70°C and then a further 400 ml of distilled water at 70°C was added. The mash was maintained at 70°C for 1 h. Finally, the temperature was increased to 79°C for 10 min and then cooled to 20°C and total weight was adjusted to 1,800 g.

3.4 Wort and beer analysis

Color, pH, Free Amino Nitrogen (FAN), turbidity, and bitterness were determined by the method of EBC (EBC, 1987). Total sugar was determined according to AOAC method no.974.06 while glucose, maltose, fructose and sucrose were assessed following AOAC method no.977.20 (AOAC, 2000). Reducing sugar was determined using 3,5-dinitrosalicylic acid (DNS) method (Miller, 1959). Extract content of the wort was calculated from the specific gravity by referring to the EBC "Table related to determination of wort sugars" (EBC, 1987). Brix was detected by hand refractometer. The pH value of wort and beer was determined by pH-meter. Alcohol was determined by Ebulliometric analysis (Zoecklein, Fugelsang, Gump, & Nury, 1989). Scavenging effect was determined by the procedure of Brand-Williams, Cuvelier, and Berset (1995). Total polyphenol contents were determined by the method of Folin-Ciocalteau phenol test (Singleton & Rossi, 1965). Vitamin B1 was determined according to the method of Liu, Zhang, Liu, Luo, and Zheng (2002) with modifications proposed by Watchararpapaiboon et al., 2010. GABA was determined by the modified procedure of Kitaoka and Nakano (1969) by following Watchararpapaiboon et al. (2010) procedure.

3.5 Fermentation

Hopped wort was pitched with 5% starter (*S. cerevisiae* RSU No.10). The fermentation batch used 20 liter of cowboy bottle sealed with an airlock and stopper. The bottles were aerated by 35 L/min 240 volt Model ACO 208 0.01 Mpa (2.175 psi) oil free piston air pump from Hailea with 0.45 micron air filter for 24 hand maintained at 10°C for 15 days. Decanted beer was further incubated at 10°C for 21 days.

3.6 Filtration

The product was filtered using kieselguhr as filter aid.

3.7 Distillation

Alcohol was evaporated from beer by using vacuum laboratory distillation apparatus in order to obtain 0.5% alcohol by weight or 0.625% alcohol by volume.

3.8 Consumer acceptability test

The 4 samples of non-alcoholic beer, rice beer from wort mashing at 65°C, rice beer from wort mashing at 75°C, beer from standard congress wort, and commercial beer brand "Clausthaler", the best-known German non-alcoholic beer from the Binding Brewery in Frankfurt, were tasted by 15 assessors who have experiences and are familiar with non-alcoholic beer. Each sample was evaluated for appearance, aroma, taste, mouth-feel and overall impression. Scores were provided on a 5-point hedonic scale where 1 is extremely dislike and 5 is extremely like (Usansa, 2008).

3.9 Statistical analysis

Results are expressed as the mean values standard deviation (SD) of three separate determinations. The data were subjected to analysis of variance. Means of each group were compared and significant differences between groups were tested as significant by Duncan's new multiple range test (DMRT) when $p \leq 0.05$. All analyses were carried out using the SPSS program.

4. Results and discussions

4.1 Characteristics of wort and beer

During mashing, about 75-80% of the grist weight is dissolved, and the insoluble residue is separated with the spent grains. The major part of the extract produced in mashing consists of fermentable sugars such as maltose, glucose, sucrose, and fructose; and unfermentable remaining parts such as dextrins, proteins, gums, and inorganic substances (Kunze, 2010). In this work, the extraction yield (in %) of wort from Thai rice prepared by mashing at 65°C and 75°C was lower than standard of congress wort of EBC Criteria. However, the result corresponded to the extraction yield of 59-62% of two black Thai rice (*Oryza sativa* L. Indica) reported by Usansa et al. (2011). The lower extraction yield occurred because of the low level of α-amylase activity and β-amylase activity. In the control production using barley malt, the average extract was 79.9% (Wijngaard & Ulmer, 2005). However, according to standard EBC method for preparing standard congress wort, % extract was determined to be 61% (data not shown) which is similar to the report of Usansa et al. (2011). Therefore, the results from the Thai rice wort are both not a surprise and also acceptable. The low amount of extract content found in starchy endosperm of rice malt is different compared to barley. From this study, it was found that the amount of reducing sugar in the extracts was 9.63-9.96 g/100g. Although the extraction yield and reducing sugar in two types of wort were not different, the amount and type of sugars were significantly different. Mashing for long periods at low (65°C) temperature favoring β-amylase production was more complete and produced wort with almost 9 times higher levels of maltose than glucose (8.65/0.98). Also, mashing for shorter periods at periods at 65°C following an increase to 75°C showed that α-amylase was activated. This was also reflected by a 2X-higher content of glucose than maltose (6.70/3.26). These results confirmed that starch degradation is sensitive to alterations in mashing temperature (De Clerck, 1957; Briggs, Hough, Stevens, & Young, 1981; MacGregor et al., 1999; Evans, Collins, Eglinton, & Wilhelmson, 2005). Preparation of wort at different temperatures affected the ratio of reducing sugars (glucose and maltose). In addition, it was found that thiamine content in wort mashing at 75°C was significantly higher than at 65°C corresponding to the results of Briggs et al.(1981). They explained that some vitamins are known to be present in malt in bound forms, from which they may be liberated by enzyme action during mashing. It is possible that higher temperatures help to denature storage proteins that are bound with thiamine in the aleurone layer in rice (Tanaka, Sugimoto, Ogawa, & Kasai, 1980) and caused the change in thiamine levels. Therefore, dissolved free thiamine content was higher like the mechanism which occurs in wheat seeds during germination (Watanabe et al., 2004). It was interesting that thiamine content in wort mashing at 75°C was 94 g/100ml, which is higher than thiamine content in standard beer (60 g/100ml) (Briggs et al., 1981). This suggested that the nutrition value of the final product would be different and could be used as point of sale. For consideration of FAN content, it was indicated that action of proteases, breaking down protein to soluble free amino nitrogen, were presented at the same level due to the same temperature and time in 'protein rest'. Although the FAN content of wort was lower than standard wort (200-250 mg/L) for commercial beer production (Pongsawadi, 2002), it was similar to FAN content of wort from Thai rice reported by Usansa et al.(2011). Yeast fermentation required FAN minimally at 200-220 mg/L and soluble nitrogen to influence beer properties. Proteinase activity was active at temperatures 45-50°C. FAN and soluble nitrogen were determined as products of enzyme activity (Kunze, 2010). Total polyphenol, GABA and color was extracted and dissolved in two types of wort at the same level. For pH, it was in the standard level of congress wort for lager beer fermentation as shown in Table1.

Table 1 Characteristics of wort

Parameter (Unit)	Wort from mashing at 65°C	Wort from mashing at 75°C	European quality requirement
%Extract (g/100g)	53.60±1.05[a]	57.56±3.65[a]	Above 80[1]
Reducing sugar (g/100g)	9.63±0.60[a]	9.96±0.55[a]	Av.8.8[1](g/100ml12%wort)
Maltose (g/100g)	8.65 ±0.10[a]	3.26±0.00[b]	5.6-5.9[1](g/100ml12%wort)
Glucose (g/100g)	0.98±0.02[b]	6.70±0.05[a]	0.9-1.2 (hexoses)[1](g/100ml12%wort)
Thiamine (μg/100ml)	45±4.5[b]	94±5.0[a]	60[2]
FAN (mg/L)	112.4±12.5[a]	113.3 ±14.8[a]	200-250[3]
Total Polyphenol(mg/L)	123±1.5[a]	125±1.5[a]	73-176[3]
GABA(g/100ml)	2570±100[a]	2825±125[a]	Has not been reported
Color (°EBC)	8.69±0.15[a]	8.69±0.15[a]	5-8 (medium colored malt)[3]
pH	5.80 ±0.11[a]	5.77 ±0.15[a]	5.5-5.8[3]

Values represent mean ± standard deviation of means.
Mean values within the same row sharing the same superscript were not significantly different at $p \leq 0.05$.
[1]Kunze (2010), [2]Briggs et al. (1981), [3]Pongsawadi (2002).

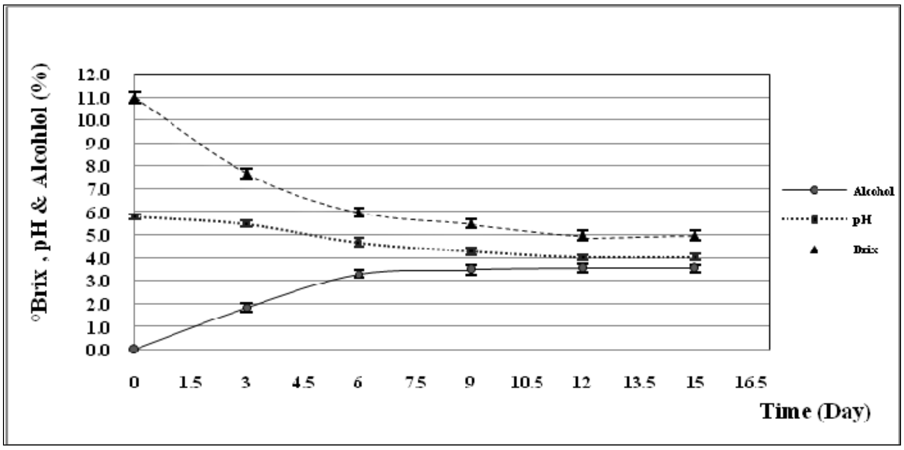


Figure 1 Change of °Brix, pH and alcohol content in wort from mashing at 65°C

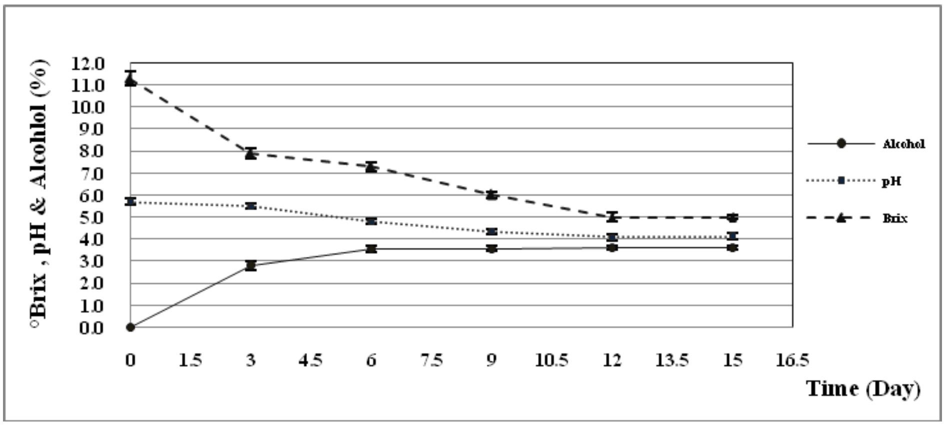


Figure 2 Change of °Brix, pH and alcohol content in wort from mashing at 75°C

As shown in Figure 1 and 2, the fermentation profiles of wort mashing at 65°C and 75°C were similar. The alcohol content gradually increased corresponding to the utilization of sugar in the respiration process. During the early stage of yeast fermentation, the remaining sugar is converted to alcohol which corresponded to the Brix decreasing. The decreasing pH was due to organic acids and hydronium ions that were released by the metabolism of cells which uptake cations into cell for osmotic pressure equilibrium (Moat & Foster, 1988).

It was found that alcohol content at the final stage of fermentation was similar at 3.56-3.60% and there was a high level of FAN content while reducing sugar was almost depleted. Additionally, the remaining reducing sugar content in beer from wort mashing at 65°C was not different from that at 75°C. The other parameters such as pH, color, and turbidity were similar as shown in Table 2.

Table 2 Characteristics of beer at the end of fermentation

Parameter (Unit)	Beer from wort mashing at 65°C	Beer from wort mashing at 75°C
Alcohol (% v/v)	3.56±0.15[a]	3.60±0.10[a]
FAN (mg/l)	59.23±13.22[a]	56.92±13.98[a]
Reducing sugar (g/100g)	0.547±0.005[a]	0.540±0.005[a]
pH	4.05±0.10[a]	4.11±0.11[a]
Color (°EBC)	9.38±0.01[a]	9.39±0.02[a]
Turbidity (EBC unit)	4.45±0.77[a]	4.38±0.47[a]

Values represent mean ± standard deviation of means.
Mean values within the same row sharing the same superscript were not significantly different at $p \leq 0.05$.

Analysis of fermented sugar remaining in two types of beers showed the composition difference as seen in Figure 3 and 4. The sugars in beer from wort mashing at 65°C were composed of glucose, sucrose, maltose, and fructose, respectively; while those from wort mashing at 75°C composed of glucose, maltose, fructose, and sucrose, respectively as arranged in order of content.

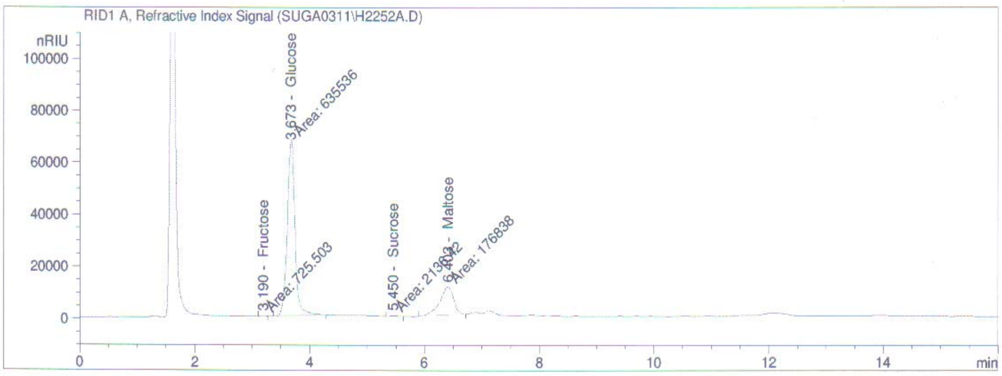


Figure 3 HPLC chromatogram of sugar in beer from wort mashing at 65°C

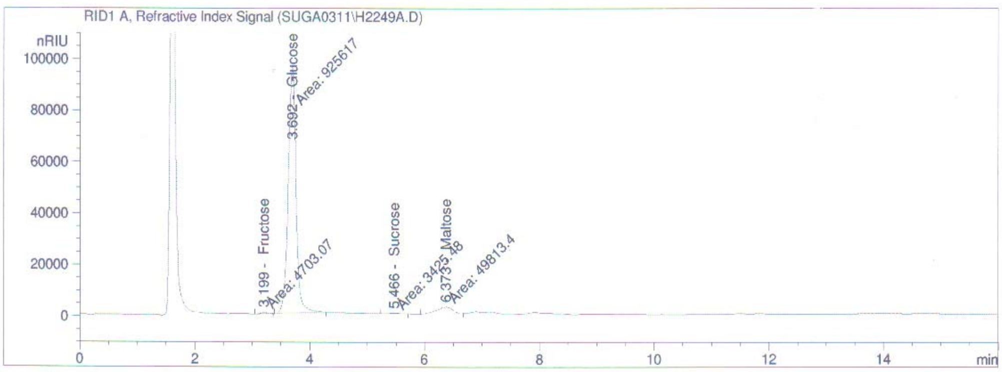


Figure 4 HPLC chromatogram of sugar in beer from wort mashing at 75°C

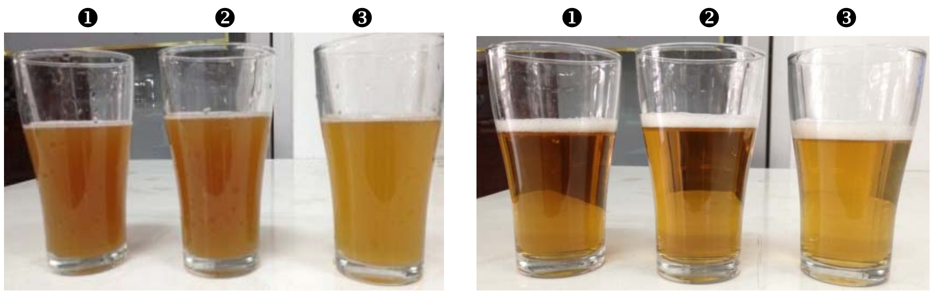
Furthermore, from Figure 3 and 4, it was observed that remaining maltose in beer from wort mashing at 65°C was higher than the maltose content observed at 75°C. In contrast, the remaining glucose in beer from wort mashing at 75°C was higher than 65°C. It could be postulated that the temperature for mashing of wort affected the type and amount of sugars. However, after ending fermentation, the alcohol content was low (3.56-3.60%) which corresponded to the report of Moonjai (2005). The research showed that sugar content from digestion of flour from rice malt was lower than from barley malt. Besides, the utilization of sugar for alcohol fermentation by yeast from rice malt was lower than sorghum and barley. Therefore, alcohol content from fermentation were limited at the same level as 3.6% which similar to 2% alcohol content in beer from rice malt while alcohol content in beer from sorghum and barley were 4.9% and 5.3% respectively as reported by Moomjai (2005). The highest reported alcohol content in beer from rice malt was only 3.98 (Salubchua, Srakeaw, & Moonjai, 2005).

After filtration of beer product through kieselguhr, the next step was evaporation to reduce the alcohol content lower than 0.5% (w/v), followed by chemical and physical analysis of mashed products at 65°C and 75°. It was found that beer from wort mashing at 75°C contained chemicals and nutrients that support the claim that beer is a healthy drink that contained higher thiamine levels and had antioxidant activity. Randhir and Shetty (2005) suggested that the antioxidant activity was linked to free soluble phenolics. Cadenas and Packer (2002) explained the role of temperature on structure of pigment and the effect of structure on changing of dissolvability including biological activity and antioxidant efficiency. The increasing of antioxidant efficiency and dissolvability of wort from mashing at 75°C as shown in Table 3 might be due to the changing of antioxidant structure at higher temperature.

Table 3 Characteristics of non-alcoholic beer

Parameter (Unit)	Beer from wort mashing at 65°C	Beer from wort mashing at 75°C	European quality requirement
Thiamine (µg/100ml)	44±5[b]	94±5[a]	60[1]
Bitter value (EBU)	18.50±0.20[a]	18.64±0.11[a]	10-55[2]
Alcohol (% v/v)	0.16±0.05[a]	0.15±0.05[a]	<0.625[3]
Total Polyphenol (mg/L)	123±1.5[a]	125±1.0[a]	73-176[2]
Scavenging effect (%)	70.5±2.7[a]	74.0±2.5[a]	Has not been reported
GABA(g/100ml)	2583±120[a]	2833±115[a]	Has not been reported
Color (°EBC)	9.38±0.01[a]	9.39±0.02[a]	8.8-26.2[2]
Turbidity (EBC unit)	2.76±0.21[a]	2.30±0.27[a]	0.1-1.0[2]

Values represent mean ± standard deviation of means.
Mean values within the same row sharing the same superscript were not significantly different at $p \leq 0.05$.
[1]Briggs, Hough, Stevens, & Young (1981), [2]Pongsawadi (2002), [3]Equivalent to 0.5% alcohol by weight (German beer institute, 2013)



(A) (B)

Figure 5 Comparison of color and turbidity between before (A) and after (B) filtration of beer
(❶=Rice beer from wort mashing at 65°C,❷= 75°C,❸=Beer from standard congress wort)

Figure 5 indicated that filtration affected the lower turbidity from level of hazy-very haze (4.38-4.45 EBC unit) to level of slightly hazy-haze (2.0-4.0 EBC unit) but did not affect the color of final product. It could be confirmed that anthocyanin, an important bio-functional compound, was not lost during filtration. The product color value was in the category of Pilsner beer as defined by the EBC scale (Kunze, 2010).

4.2 Sensory evaluation

The non-alcoholic beers were tested by 5-point Hedonic scale to evaluate 5 attributes of the products. There were 4 samples for comparison: beer from wort mashing at 65°C, 75°C, control beer prepared from barley malt, and trade beer (Clausthaler) from Germany. The results (Table 4) indicated that consumers accepted non-alcoholic beer from Thai rice produced from wort mashing at 65°C and 75°C as shown by the similar score levels of each attribute of the products compared to the control products. There were no significant differences in the 4 samples in the term of appearance, aroma, taste, mouth-feel, and overall impression. It was noticed that mouth-feel received higher score than other attributes. This result was related to dextrin content, complex sugars remaining in wort, not fermented by yeast. Mashing at 65°C and 75°C caused the starch conversions to stay in dextrins, producing a more full-bodied beer with less alcohol and agreed with Naleszkiewicz (1995). He suggested that dextrins would not contribute as much to the sweetness as they would increase the fullness of beer. When taking out the alcohol, it was good to give the beer a little more body. The more dextrins there were the better. Therefore, this research confirmed that beer products from Thai rice are acceptable and have a potential for commercial production.

Table 4 Sensory evaluation of non-alcoholic beer

Sample	Attributes				
	Appearence	Aroma	Taste	Mouth-feel	Overall impression
Rice beer from wort mashing at 65°C	3.55±0.98[a]	3.37±0.64[a]	3.20±0.32[a]	3.95±0.84[a]	3.56±0.67[a]
Rice beer from wort mashing at 75°C	3.47±0.92[a]	3.42±0.66[a]	3.33±0.87[a]	3.96±0.94[a]	3.43±0.82[a]
Beer from standard congress wort	3.87±0.91[a]	3.25±0.80[a]	3.61±0.88[a]	3.97±0.83[a]	3.80±0.99[a]
Clausthaler	3.75±0.75[a]	3.18±0.75[a]	3.62±0.85[a]	4.00±0.75[a]	3.75±0.88[a]

Values represent mean ± standard deviation of means.
Mean values within the same column sharing the same superscript were not significantly different at $p \leq 0.05$.

5. Conclusion

Rice wort qualities from wort mashing at 65°C and wort mashing at 75°C showed the same level of % extract, reducing sugar, FAN, color and pH. The fermentation profiles from wort mashing at 65°C and 75°C were not different. The decreasing of Brix and pH were found whereas alcohol content was increased during the fermentation period. The limitation of reducing sugar content and FAN affected yeast metabolism and limited the level of alcohol content. There was no difference of physical properties of beer from wort mashing at 65°C and wort mashing at 75°C in color and turbidity values. There was no difference of chemical properties of beer at final stage of fermentation beer from wort mashing at 65°C and 75°C in pH, color, turbidity, FAN, alcohol and reducing sugar. Non-alcoholic beer from wort mashing at 65°C and 75°C had the same content in color, turbidity, bitterness, % alcohol. Bio-functional compounds in beer from wort mashing at 65°C and 75°C indicated that phenolics, % radical scavenging and GABA content were similar whereas, beer from wort mashing at 75°C had 2.14 times higher thiamine content. Filtration through the kieselguhr had resulted in a significant reduction inturbidity while it did not affect color. The final turbidity of beer was 2.30-2.76 °EBC. For sensory evaluation, the assessors accepted all attributes in level of “moderately to like” similar to control beer, suggests that non-alcoholic beer from Thai rice has potential for use in brewing. It can be concluded that non-alcoholic beer prepared from wort mashing at 75°C was a good healthy beverage since it contained high levels of thiamine and antioxidant while it contained low sugar content.

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