

THE OPTIMIZATION OF MAIZE BREWING PROCESS AND BARLEY SUPPLEMENTATION FOR THE NEW BEER PRODUCTION

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Abstract

Beer is generally made from barley which has a high amount of gluten protein. Gluten is involved in celiac disease. This research aims to optimize the brewing process using maize, a gluten-free grain, using a new mashing program to create the wort from maize malt to increase the value of maize and make a new beer product. The optimum malting condition is when maize is steeped at 30°C for 24 h and germinated at 30°C for five days. In this condition, the highest value of α - and β -amylases was 39.76 U/mg and 32.88 U/mg, respectively. The results indicated that the use of a 4-step infusion mashing program completely hydrolyzed the starch and protein degradation. The nitrogenous substances were similar when compared with barley malt where the Kolbach index and FAN (free-amino nitrogen) were 40.49% and 239.40 mg/L, respectively. These results indicated that the maize is good for brewing. The wort extracted from 100% maize malt, 100% barley malt, and 50% maize malt with 50% barley malt were appropriate for brewing in a 50 L pilot brewing plant. The wort contained 139.60, 132.81, and 136.53 g/L of total fermentable sugar, respectively. Simultaneously, the FAN and extract contents from 100% maize malt were 348.99 mg/L and 14.74°P, respectively. Furthermore, the sensory test of maize beer judged it as drinkable and preferable to the next glass. The results obtained from this research demonstrated the suitability of maize for brewing technology as a raw material with an acceptable quality.

Keywords: Maize malt beer, malting, mashing, fermentation, sensory analysis

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Introduction

Beer is the most consumed alcoholic beverage in the world. The worldwide beer production is approximately 2 billion hectoliters per year (Amienyo and Azapagic, 2016). The global beer market was valued at nearly \$600 billion in 2017 (Sinha, 2018) and is increasing. Beer generally utilizes malted grain, mostly barley and wheat, which provides fermentable sugars and protein in the wort. The use of gluten ingredients in brewing may cause harm to consumers who have celiac disease. Celiac disease is defined as an autoimmune disorder that causes the immune system to respond to the body's ingestion of the protein in certain grains (Bower *et al.*, 2006).

Celiac disease has been known for more than 50 years (Hager *et al.*, 2014). The primary mechanism involved in celiac disease is related to an inappropriate adaptive immune response to gluten-derived peptides (Parzanese *et al.*, 2017). Damage is caused to susceptible patients because the immune system in the human intestine builds antibodies against the gluten protein found in wheat (gliadin), barley (hordein), and rye (secalin) (Bower *et al.*, 2006).

Celiac is one of the common chronic health diseases in Western countries. Recent studies have estimated that approximately 1% of the worldwide population suffers from this disease (Lebwohl *et al.*, 2015; Malalgoda and Simsek, 2017). A gluten-free diet is necessarily beneficial for a patient who is diagnosed with celiac disease. However, the availability of gluten-free food is not usually found in commonly consumed products. The rising incidence of obesity, diabetes, celiac disease, and other diagnosed food intolerances, as well as the growing consumer awareness to address these conditions, have expanded the market for the manufacture of customized allergen-free products. The market for gluten-free products was estimated at more than \$15 billion in 2016 (Niland and Cash, 2018). Beer is a cereal-derived product that is eliminated from the diet of a celiac patient. A significant part of the population, including celiac patients, would consider beer as an inherent part of their diet (Harasym and Podeszwa, 2015). The gluten-free beer market

is still perceived as being less commercially viable by the big breweries. However, the increase of market value could encourage the brewing industry and scientists to pay more attention to gluten-free beer.

For brewing, barley and wheat, which contain significant amounts of gluten, are most widely used. Therefore, beer is unsuitable for celiac disease patients. To eliminate the gluten in beer, several techniques have been applied, for example, enzymatic treatment (Hartmann *et al.*, 2006; Caputo *et al.*, 2010; Van Landschoot, 2011; Guerdrum and Bamforth, 2012) and precipitation of hordeins (Dostálek *et al.*, 2006). In addition, the possibility of brewing beer from alternative cereals or pseudo-cereal material, such as rice (Usansa *et al.*, 2009; Mayer *et al.*, 2016), sorghum (Figueroa, 1995; Ogbonna *et al.*, 2004; Djoulde *et al.*, 2013), and corn grist (Poreda *et al.*, 2014), has been studied.

In the current study, the authors intended to develop gluten-free beer in brewhouse conditions. However, we found that both rice and sorghum are of low quality due to the low protein content (Agu *et al.*, 2012; Kongkaew *et al.*, 2012; Wrigley *et al.*, 2016). Protein degradation is insufficient and may affect fermentation and leads to poor sensory characteristics. Maize is most widely used in brewing as an adjunct grain to lighten the beer's body, color, and flavor (Lewis and Young, 2002; Stewart and Priest, 2006; Bamforth, 2016). 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 higher levels of protein and fat (Bamforth, 2016). Maize malts have the efficiency to be used for brewing as a source of carbohydrate and protein in beer. The optimized brewing process could produce a new lager beer from maize.

This study demonstrated the potential of maize for brewing the new gluten-free beer. This research's objective was to optimize the maize malting conditions to produce the wort from mashing 100% maize malt. On the other hand, the addition of barley malt at different ratios was carried out to prove the viability of maize malt when combined with barley

malt. Finally, the finished beer quality was analyzed.

Materials and Methods

Raw Materials and Yeast Strain

The maize, Suwan 5, was purchased from the National Corn and Sorghum Research Center, Thailand. The Pilsner barley malt (Weyermann Malting Company, Bamberg, Germany) and Citra Hops pellets type 90 (10-14% acid) were purchased from Yakima Chief Hops, Yakima, WA, USA. The lager yeast, *Saccharomyces pastorianus*, strain Saflager S-189 for bottom fermentation, which is widely used in the brewing industry, was purchased from Fermentis, Marcq-en-Baroeul, France.

Maize Properties

A proximate analysis of maize, including moisture (Method 945.15), fiber (Method 962.09), total nitrogen (Method 984.13), fat (Method 920.39), and ash (Method 923.03), was carried out according to the Association of Official Analytical Chemists (AOAC, 1990). Total carbohydrate content was calculated by difference after analysis of all the other item methods in the proximate analysis (AOAC, 1990). Thousand grain weight (European Brewing Convention (EBC) 4.4) and germination percentage of maize were determined according to the European Brewing Convention (1998). The gelatinization temperature of maize was carried out using a DSC 7 differential scanning calorimetry (Perkin Elmer Corp, Norwalk, CT, USA). The gluten content was detected using an AOAC-certified gliadin R5 ELISA kit (Veratox, Neogen Food Safety, Lansing, MI, USA). Each experiment was duplicated.

Influences of Malting Condition on Malt Quality

The malting process was manipulated by using 800 g of maize grain in metal mash chambers (20×20×40 cm). All chambers of maize were washed with reverse osmosis water before steeping at 30°C for 24, 36, and 48 h; the water to grain ratio was 3:1 (w/w). The steeping degree of maize was controlled by

re-steeping or spraying water. After this equilibration, maize boxes were put in a germination room with a relative humidity of 95±5% and the temperature was controlled at 20, 25, 30, 35, and 40°C, separately. After the germination, the sprouted maize was kilned in a hot air oven at 50°C for 24 h. Then, roots and shoots were removed.

Analysis of the maize malt including an extract of malt (congress malt) and FAN was performed according to EBC 4.5.1 and EBC 8.10, respectively (European Brewing Convention, 1998). The α - and β -amylase activities were analyzed according to Usansa *et al.* (2009). 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 25°C 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×g (320 R Universal centrifuge, Andreas Hettich GmbH & Co.KG, Tuttlingen, Germany) 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. 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 the addition of 1 mL of 3-5, dinitrosalicylic acid reagent. For the β -amylase assay, the reaction was constructed in the same procedure as the α -amylase but the 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 the reaction.

The protease activity was determined by adding 100 μL of crude extract with 150 μL NaHCO_3 0.5% (w/v) and 250 μL azocasein solution 2.5% (w/v). The assays were then shaken well to ensure thorough mixing and incubated in a constant temperature water bath at 37°C for 30 min. The control sample consisted of 250 μL NaHCO_3 0.5% and 250 μL azocasein solution. Assays were stopped by the addition of 1 mL of 5% TCA, shaken, and left at room temperature for 10 min and then centrifuged at 10,000×g

(Denville 260D Microcentrifuge, Harvard Bioscience, Inc., Holliston, MA, USA) for 5 minutes to yield a yellow-orange supernatant containing the cleaved azo dye. One mL of supernatant from each assay was transferred to a 1 mL cuvette, then the absorbance at 440 nm was analyzed using a T80+spectrophotometer (PG Instruments Ltd., Lutterworth, UK). Each experiment was duplicated.

Wort Preparation and Beer Fermentation

The maize malt from the optimum malting condition was chosen for the wort preparation. The maize malt was kilned in a hot air oven at 50°C for 24 h, and then roots and shoots were removed. The malt was stored in a plastic bag until use. The 3 types of wort consisted of 100% maize malt, 50% maize malt supplemented with 50% barley malt, and 100% barley malt. These malts were mashed in different mashing profiles, the wort from the 100% maize malt and 100% barley being mashed by the infusion method. The process for the 50% maize malt supplemented with 50% barley malt was conducted by the decoction method. The malts were ground by using a Bühler Miag laboratory disc mill 0.5 mm (Bühler AG, Uzwil, Switzerland). For the 100% maize malt wort, it was then mixed with water at 45°C at a ratio 1:5; 9 kg of maize malt was mashed with 45 L water. The mashing pH was adjusted between 5.0 and 5.2 using lactic acid 1 M. The mashing process involved a 4-step program starting at 45°C within 30 min in a 50 L brewing plant (Softech, Bangkok, Thailand). Next, the temperature was increased to 62°C within 10 min and held for 30 min, then 72°C within 10 min and held for 30 min. Following this, it was increased to 100°C for 20 min.

For the supplemented wort consisting of 50% maize malt and 50% barley malt, 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 (w/w) 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 within 10 min) and held for 30 min. Then, the temperature was increased to 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 the 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 (the 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.

The mashing process of the 100% barley malt wort took 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.

With the mash transfer to the lauter tun, after separation of the spent grains and the wort, the wort was boiled for 60 min at 100°C; three-quarters of the hop pellets were added after 15 min, and one-quarter after 45 min to obtain the final beer bitterness for 30 BU (mg of bitter substance per liter of wort). The sterilized wort was transferred to the whirlpool for clarification and the wort was cooled to 14°C.

After cooling the wort (35 L) down to 14°C, bottom-fermenting yeast was pitched at 1.4×10^7 cells/mL at 14°C and the fermentation process was started. The filtered air was injected into a fermenter for 30 min to achieve adequate oxygenation. The fermentation process was carried out in a 50 L fermenter (Softech, Thailand). The properties of the wort including the extract of malt (congress malt) and FAN were performed according to EBC 4.5.1 and EBC 8.10, respectively (European Brewing Convention, 1998). Each experiment was duplicated.

Fermentable Sugar and Ethanol in Wort and Beer

Fermentable sugars in the fermented wort comprising glucose, fructose, maltose, and maltotriose, were analyzed using HPLC with a refractive index detector (Agilent 1200, Agilent Technologies, Santa Clara, CA, USA). Samples were diluted with deionized water at a ratio of 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 300×7.8 mm (Phenomenex, Torrance,

CA, USA). The temperature of the column and detector were set at 55°C and 35°C, respectively. The quantities of fermentable sugar and ethanol in the samples were calculated using the standard equation.

Volatile Compounds and Higher Alcohols in Beer

Volatile compounds and higher alcohols in the finished beer were measured using a GC model Agilent 7890A coupled with a mass detector model 7000 Triple Quad GC/MS (Agilent, USA) by the solid phase micro-extraction technique. One mL of the diluted sample was filtered and injected into a piercing septum vial. Samples were heated to 70°C and agitated continuously at 600 rpm for 20 min. The volatile compounds of the samples were collected using a polydimethylsiloxane fiber syringe (Supelco Inc., Bellefonte, PA, USA). The needle can pierce the septum of a vial containing the sample. After that, the fiber was exposed to the headspace of the sample (20 mm above the sample surface) for 20 min. All analytes that were adsorbed on the fiber were desorbed by piercing the GC inlet septum for 5 min.

The standard mixes of 6 levels (10, 20, 40, 60, 80, and 100 ppm) consisting of dimethyl sulfide, acetaldehyde, propanol, isoamyl alcohol, butanol, ethyl acetate, isoamyl acetate, 2,3-butanedione, 2,3-pentanedione, 2,3-hexanedione, 2-phenylethyl acetate, octanoic acid, ethyl caproate, ethyl caprate, and ethyl caprylate were prepared using 40% ethanol as a diluted solution. All samples and standards were injected and separated on a DBWAX column, 60 m × 0.25 mm I.D. and 0.25 µm film thickness (Agilent, USA). The column temperature-program was 35°C for 5 min and increased to 230°C by 6°C per min (running time 42.5 min). The mass detector condition was an electronic impact, EI0 mode at -70 eV. The source temperature was 220°C, and the scanning rate 1 scan/s. The mass acquisition range was 45-170. The carrier gas was helium at 1.0 mL/min. Identification of the volatile components of the beers was carried out based on a comparison of their GC retention times and mass spectra with authentic standards from the NIST Mass Spectral Search

Program for the NIST/EPA/NIH Mass Spectral Library version 2.0 (National Institute of Standards and Technology, Gaithersburg, MD, USA).

Beer foam Stability

The samples were placed into the graduated cylinder. The foam stability was measured as the seconds needed for the foam to collapse over a distance of 30 mm. The measurement started 10 mm below the rim of the cylinder. The times it took for the foam to collapse over the distances of 10, 20, and 30 mm were displayed as a result.

Sensory Analyses of Beer

Sensory tests of the beers were performed using the hedonic test (Bamforth, 2016). Sensory evaluation of the beers was carried out by a trained, 16-member tasting panel (women and men, 20 to 60 years old). The 5 attributes assessed for the taste were appearance, aroma, flavor, mouth-feel, and overall impression. The hedonic scale (5 levels) was designed as 0 to 5 scores for evaluating the aroma, appearance, flavor 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 preferable to the next glass, drinkable and preferable to the next glass, good, very good, and excellent, respectively.

Statistical Analysis

The statistical analysis was carried out by using SPSS version 16.0 for Windows. All chemical experiments were analyzed in duplicate. Analysis of variance (ANOVA) and means comparison by Duncan's multiple range test (DMRT) were used to determine differences between mean at $p < 0.05$.

Results and Discussion

Maize Properties

Suwan 5 is a single-cross hybrid maize, a source for high-yielding and downy mildew-resistant crops. It had good agronomic traits and also produced high fresh and dry weights

(Aekatasanawan *et al.*, 1994). The high-yielding property of this maize explains its very high percentage of germination (96%). The maize contained 9.01% of protein, which was similar to that of the barley (9-11%). Gluten was not present in the maize. This result confirmed the ability of maize to produce gluten-free beer. As shown in Table 1, the carbohydrate content of the maize was 73.87%, which was similar to the barley (75-79%). It was indicated that maize is a potential carbohydrate source for brewing.

On the other hand, the maize contained 3.9% of fat which is higher than the barley (1.8%). However, during the germination of the maize, the fat content decreased according to the germination (Ingle *et al.*, 1964). The bulk of the fat reserve of the seed was progressively depleted over the 5-day germinating period from 10 mg/part to 3 mg/part. The changing of the lipid content during the malting stage was also confirmed by Bravi *et al.* (2012). The monitoring of the total lipid content during the malting process showed significant decreases moving from the barley to the malt. This variety of maize has benefits for malting and brewing from its properties.

The Influences of Germination Conditions on Malt Quality

The α - and β -amylase Activities from Crude Enzyme Extraction

The longer germination time led to higher enzyme activity, as shown in Figure 1. The enzyme activities were slightly increased from the first 2 days; afterwards, the α - and β -

amylases were dramatically developed to their highest values. In this study, maize steeped for 24 h displayed the highest enzyme activity, and the maximum value was reached at day five at 30°C, followed at 20 and 25°C (Figures 1(a) and 1(b)). At higher temperatures of 35 and 40°C, the enzyme activity was not significantly changed due to the inhibition of germination at a high temperature. Riley (1981) demonstrated that 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 the germinating maize seeds to high temperature. The maximum level of α - and β -amylase activities in the maize was 39.76 and 32.88 U/mg, respectively. The amylase activities of the maize malt were higher than the rice malt which was reported by Usansa *et al.* (2009), when the maximum α -amylase and β -amylase activities found in the germinated PT60 rice steeped for 24 h and germinated for 5 days at 30°C were 13.40 and 5.45 u/mg protein, respectively.

Due to the anaerobic condition, when the grains were steeped for a long time, the maize was affected, which led to the reduction of the germination rate along the steeping time. Following along the steeping time, the enzyme activity reduced when the maize was steeped for 36 h (Figure 1(c) and 1(d)) and was lowest when the maize was steeped for 2 days (Figure

Table 1. The proximate analysis result of Suwan 5 and Pilsner malt. The data was expressed in percentage of dry weight

Nutrients	Chemical compositions	
	Suwan 5	Pilsner malt
Moisture (%)	8.79 $\pm$ 0.09	5.00 $\pm$ 0.02
Crude protein (N=6.25) (%)	9.01 $\pm$ 0.14	10.50 $\pm$ 1.00
Fat (%)	3.90 $\pm$ 0.23	1.78 $\pm$ 0.17
Crude fiber (%)	2.04 $\pm$ 0.05	3.47 $\pm$ 0.41
Carbohydrate (%)	73.87 $\pm$ 1.36	76.24 $\pm$ 0.98
Gluten (ppm)	<5.22 $\pm$ 0.05	>80.00 $\pm$ 0.38
Ash (%)	2.17 $\pm$ 0.12	0.93 $\pm$ 0.09
Percentage of germination (%)	96	ND
Thousand grain weight (g)	343.77 $\pm$ 4.09	42.07 $\pm$ 0.78

1(a) and 1(f)). When prolonging the steeping time, the maximum amylase activities were reached in a longer time.

Effect of Malting Conditions on Protease Activity

The protease activity significantly increased through the germination time. The optimum protease activity for germination at 30°C in all conditions of the steeping time for 24, 36, and 48 h can be obtained from Figures 2(a), 2(b), and 2(c), respectively. A high temperature (over 35°C) for maize would be expected to

retard the metabolic rate. In the embryos of maize, heat shock markedly activated protein synthesis during the early stages of germination, and heat shock proteins' synthesis was induced simultaneously with the synthesis of the bulk of the proteins produced by the embryo tissues before heat shock (Riley, 1981). In addition, Azarkovich (2016) reported the effect of heat shock on protein synthesis in the seed embryos of various grasses during seed development and germination. As a consequence, the physiology of the seed was significantly changed. The study concluded that this could be a manifestation

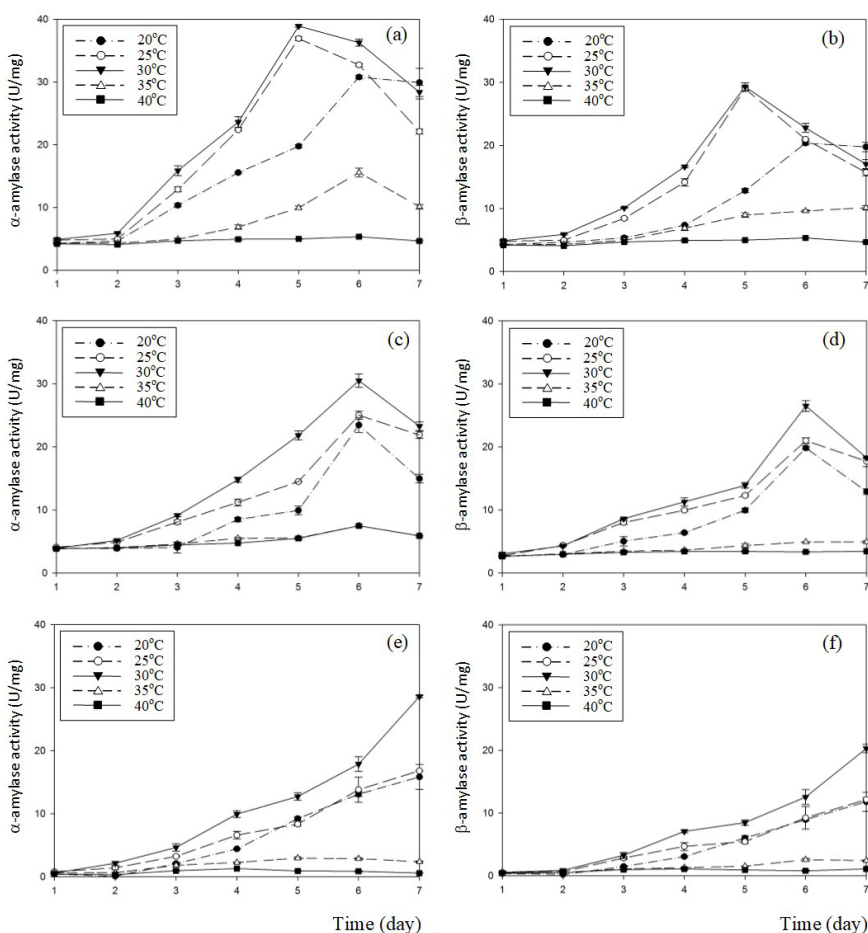


Figure 1. The time course of α - and β -amylases production in germinated maize at the different temperatures after the various steeping times: (a) and (b) steeping 24 h; (c) and (d) steeping time 36 h; (e) and (f) steeping time 48 h. (a), (c), and (e) α -amylase activity; (b), (d), and (f) β -amylase activity. Steeping temperature was at 30°C

of additional molecular mechanisms improving embryo tolerance to unfavorable environmental conditions and, as a result, their viability.

The highest amount was found in a sample germinated at 30°C. However, based on the steeping time and germination time, the proteolytic activity was changed. This result indicated that, on the fifth day of germination, the maize steeped for 24 h was suitable for enzymatic production.

Nitrogenous Substances

The relationship of the soluble nitrogen percentage on the total nitrogen percentage in terms of the Kolbach index was analyzed. As

can be observed in Figures 3(a), 3(c), and 3(f), the Kolbach index was increased during germination time. The results indicated that the Kolbach index and the FAN were increased by increasing the germination time and temperature (Figure 3). Two of these parameters changed during the development of enzymatic activity, especially protease activity. The steeping regime of 24 h and germination at 30°C at the fifth day of germination provided the highest amount of those nitrogenous components in the maize malt. The FAN content in the congress mash is confirmed to be a good index for potential yeast growth and fermentation efficiency. The FAN obtained from the maize malt was in the range of 200- 250 mg/ mL, while the rice malt wort that was reported from Ceccaroni *et al.* (2019) showed a lower FAN content (188 mg/L) when compared with the maize malt. Therefore, the maize malt was suitable to be used in beer production.

Extract Content from Congress Mashing

The 4-step infusion mashing program for the hydrolysis of starch and protein was mentioned previously. The extract content of the congress mashing from each germination condition (1st-7th day) was analyzed. The extract of the maize malt was performed by the congress mashing method according to EBC 4.5.1 (European Brewing Convention, 1998). Figure 4 showed that, through the germination time, the extract content was increased from the first day to the 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 had a lower value of extract content. Since the development of the amylase enzymes during germination was at a differential steeping time, the results explained the influences of enzyme activity on the concentration of the extract. The increase of enzyme activity correlates with the increasing of the extract content. Moreover, the extract was significantly affected by the germination time; particularly, at 24 h steeping time, the extract yield was increased with the 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

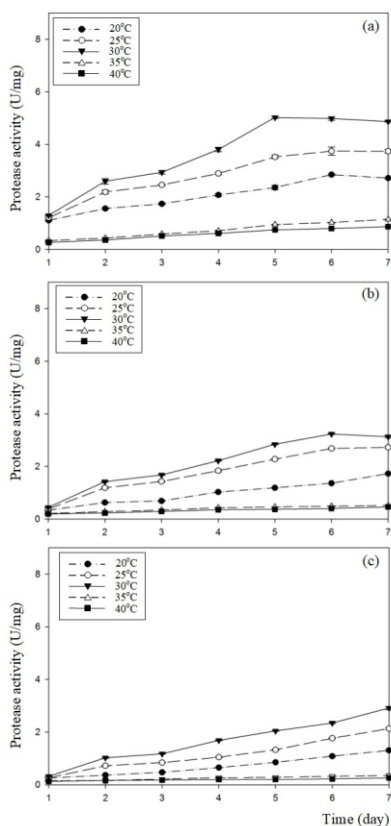


Figure 2. The time course of protease activity in germinated maize at the different temperatures after the various steeping times: (a) steeping time 24 h; (b) steeping time 36 h; (c) steeping time 48 h. Steeping temperature was at 30°C

lower than that of 30°C. The highest value of the extract from the maize malt was 61.5%, while the extract of barley was in the range of 79.3% to 81.4%. The explanation for this lower extract yield is that the gelatinization temperature of maize starch was higher than 72°C (the gelatinization temperature range of maize was in the 70.6 to 79.6°C range and the maximum was at 75.6°C), the saccharification temperature in congress mashing. These obtained results are similar to a report by Pelembe *et al.* (2004) that the low extract content of sorghum malt was caused by the starch gelatinization

and hydrolysis. Thus, the low amount of extract content found in the maize malt was reasonable and acceptable.

In addition, the concentration of reducing sugar presented in the congress wort that corresponded with the extract yield was logically increased with the germination time and was affected by the germination temperature. The results revealed that the steeping time, steeping temperature, germination time, and germination temperature were the important factors for malt quality. The best values of enzyme activities, FAN, Kolbach

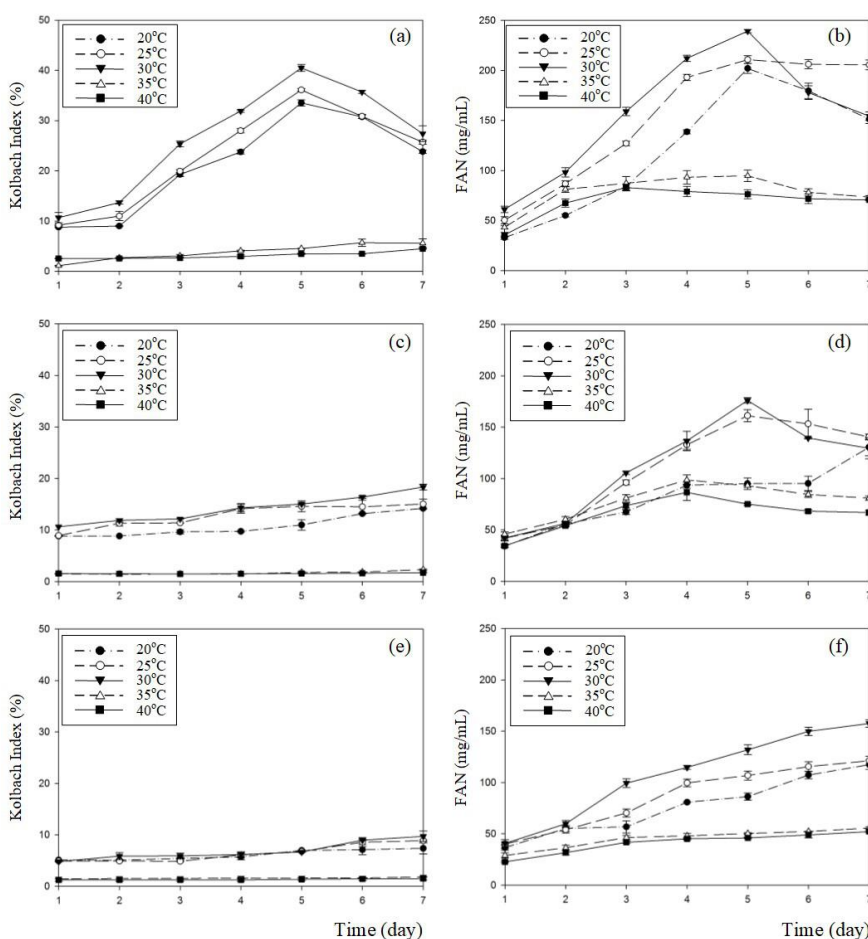


Figure 3. The time course of Kolbach index and FAN in germinated maize at the different temperatures after the various steeping times: (a) and (b) steeping time 24 h; (c) and (d) steeping time 36 h; (e) and (f) steeping time 48 h. (a), (c), and (e) α -amylase activity; (b), (d), and (f) β -amylase activity. Steeping temperature was at 30°C

index, extract content, and reducing sugar were present in the maize that was steeped at 30°C for 24 h, and germinated at 30°C for 5 days. Therefore, these malting conditions were chosen for the next experiments.

Wort Preparation from Maize Malt and Barley Malt

The quality of the maize malt wort and barley malt wort was analyzed and is illustrated in Table 2. The FAN in the maize malt was 349 mg/mL and was higher than the barley malt (290.33 mg/mL). Therefore, when increasing the proportion of maize malt, the FAN was increased. When the FAN content of the maize malt was compared with that of another malt, the maize malt showed a worthy value. Igyor *et al.* (2001) studied the effect of the malting temperature and mashing methods on sorghum wort composition. The peak of FAN in the wort from sorghum malt was 177 mg/mL, whereas, the beer brewed from 100% buckwheat malt showed the maximum amount of wort was 210 mg/mL (Phiarais *et al.*, 2005). The extract amount of each proportion was similar at 14.74°P, the higher concentration of extract content when compared with the wort from the congress mash due to the mashing profile when the temperature reaches the saccharification point.

The wort contained a high concentration of maltose and maltotriose, which were formed during the malting stage. Also, the glucose and sucrose components were present in the wort. The maltose in the 100% maize malt wort is slightly higher than in the wort of the 100% barley malt. In contrast, the maltotriose component in the maize malt wort is lower than the barley malt wort. Marconi *et al.* (2017) brewed beer from rice malt wort; the results showed a lower total sugar profile at a different mashing process when compared

with the maize malt wort. Di Ghionno *et al.* (2017) studied the quality attributes of gluten-free beer from malted and unmalted teff (*Eragrostis tef* (zucc.) trotter). The standard quality of the wort was assessed following the European Brewing Convention. It was shown that the original extract (°P), FAN (mg/L), and fermentable sugars of the teff wort were lower than for the maize wort.

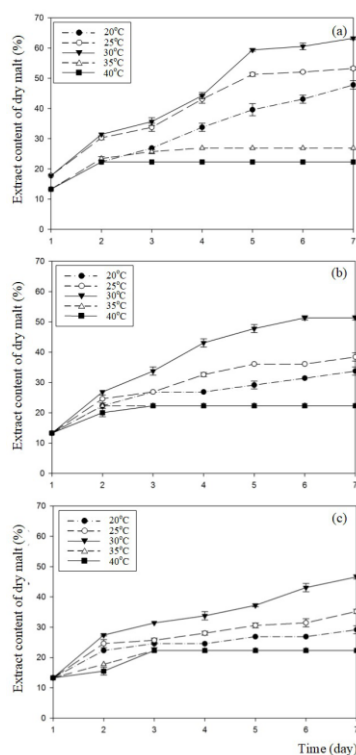


Figure 4. The time course of extract content in the congress wort of germinated maize at the different temperatures after the various steeping times: (a) steeping time 24 h; (b) steeping time 36 h; (c) steeping time 48h. Steeping temperature was at 30°C

Table 2. Wort analysis from maize malt mashing

Ratio (Maize:barley)	FAN (mg/L)	Extract content (°P)	Fermentable sugar (g/L)				
			Maltotriose	Maltose	Glucose	Fructose	Total
100:0	348.99±0.41	14.74	39.74±0.78	68.32±1.63	29.54±0.95	2.00±0.17	139.60
50:50	319.02±1.23	14.74	40.32±0.24	68.43±0.42	27.62±1.03	0.16±0.02	136.53
0:100	290.33±0.43	14.74	41.90±0.61	64.32±1.12	26.36±0.63	2.23±0.09	132.81

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

Beer Fermentation and Volatile Compounds of the Final beers

The FAN, extract content ($^{\circ}\text{P}$), and reducing sugar as a function of time are shown in Figure 5(a), 5(b), and 5(c). These ingredients were rapidly consumed from 0 to 72 h, and they were kept constant afterwards. The explanation for this tendency is the utilization of yeast along with fermentation. In addition, the fermentable sugars were utilized and

decreased during fermentation. Generally, glucose and fructose fell considerably and were exhausted by 96 h of the fermentation period. The reason is that the 2 sugars were monosaccharides and were consumed before the maltose and maltotriose. Maltose and maltotriose were slightly reduced at the beginning of fermentation at 48 h. Afterwards, the significant utilization of the disaccharide and trisaccharide occurred at 48-216 h (Figure 6). In the same way, the extract yield and reducing sugar also dropped accordingly.

The change of pH during fermentation was caused by the release of CO_2 , the ammonium ions uptake, and organic acid excretion of yeast cells under anaerobic conditions. This result agreed with Coote and Kirsop (1979) when they investigated the factors responsible for the decrease in pH

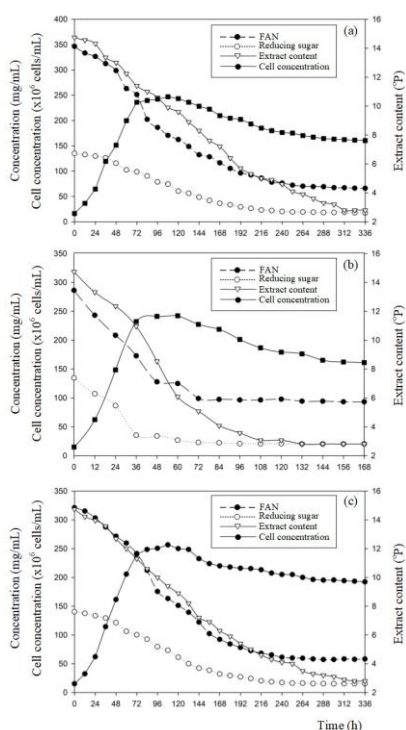


Figure 5. The time- course of FAN, reducing sugar, extract content and viable cell count during fermentation of 100% maize malt (a), 100% barley malt (b), and 50% supplemented maize malt (c)

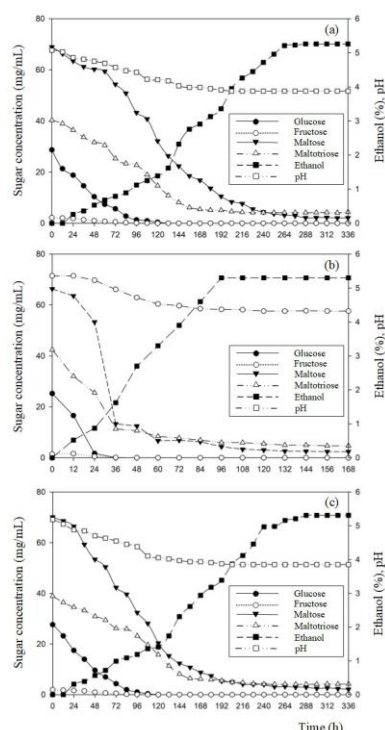


Figure 6. The time-course of fermentable sugars, ethanol, and pH during fermentation of 100% maize malt (a), 100% barley malt (b), and 50% supplemented maize malt (c)

during beer fermentation.

As illustrated in Figure 6(a), 6(b), and 6(c), the analyzed ethanol was increased whereas the fermentable sugars decreased. Furthermore, the pH value went down as well as the ethanol accumulation. These results reasonably corresponded with the changing trends in many yeast strains (Mohammadi *et al.*, 2011).

The volatile compounds in beer which consist of higher alcohol or fusel alcohol, ester compounds, and free fatty acids were analyzed as summarized in Table 3. Isoamyl alcohol is the most quantitatively important compound for flavor in the higher- alcohol group. It influences drinkability because the beer flavor becomes heavier if the isoamyl alcohol concentration increases (Olaniran *et al.*, 2017). Isoamyl alcohol was in the range of 45- 60 ppm, which was lower than the standard beer (60 to 90 ppm) (Kunze, 2004). 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.

Esters are the secondary products and the most important compounds influencing beer flavor. Ethyl acetate, isoamyl acetate ethyl caproate, ethyl caprylate, ethyl caprate, and 2-phenylethyl acetate are represented in the ester group in brewing. In bottom fermentation, beers contain esters up to 60 ppm (Kunze, 2004). In this study, all ester compounds were in the range of standard beer. Ethyl caproate concentrations in beers are from 17 to 25 ppm. The presence of 5 mg/L of hexanoic acid (ethyl caproate) was characterized as the cheesy,

goaty, and sweaty flavor (Šmogrovičová and Dömény, 1999). The final beers did not contain aldehyde, diacetyl, and sulfur compounds which impart off-flavors. This result indicated that the beers studied did not have any off-flavors.

Sensory Evaluation of Final Beer

Three kinds of experimental beer were used for this test (Table 5). The appearance of the beer was evaluated according to the clarity, color, bubbles, or head formation. The sensory evaluation was undertaken by trained panelists. In beer made from 100% maize malt, the average score of appearance was 2.75 (normal to like). When the ratio of barley malt was increased, the score of the appearance increased as well. The highest score presented in 100% barley malt beer was 3.98 (like very much). Moreover, the color of the beer is in a range of 14-20 EBC units. The barley beer was reddish-gold in color, whilst the maize beer displayed brown and the 50% maize beer presented deep amber. When increasing the amount of maize malt, the color of the beer was darker due to its high-temperature mashing process which caused a caramelization reaction. The beer had a regular foam stability and clarity, and the judges scored it from 2.75 to 3.98. Following 7 to 9 min there was a thin head that kept going until the end (Table 4).

The maize beer had a stronger mead aroma than the barley beer, which led to the stronger honey aroma. The aromas from the 2-treatment 50% and 100% maize malt were not significantly different and scored 2.38 and 2.25, respectively, and these beers were

Table 3. Volatile compounds in final beers

Compound	Concentration (ppm)		
	100% barley malt	100% maize malt	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

The different superscript letter between the columns was a significant difference at $p \leq 0.05$

Table 4. Beer foam stability and color of final beers

Properties	100% Barley malt	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

Table 5. Sensory evaluation of final beers

Properties	100% Barley malt	100% Maize malt	50% Maize malt
Appearance	3.25±0.41	2.75±0.31	3.00±0.33
Aroma	3.89±0.23 ^a	2.25 ±0.25 ^b	2.13±0.30 ^b
Flavor	3.75±0.31 ^a	2.25 ± 0.31 ^b	3.25±0.31 ^a
Mouth-feel	3.89±0.30 ^a	2.50 ±0.33 ^b	2.89±0.23 ^{ab}
Overall	4.00±0.27 ^a	2.38 ±0.18 ^b	3.38±0.16 ^a

Mean values of 16 replications of analysis ± standard deviation. The different superscript letter between the columns was a significant difference at $p \leq 0.05$

acceptable. The Pilsner beer was most favorable at 3.9 (like to like very much). The high ratio of barley malt showed a high score of favorability. After evaluating the beers' appearance and aroma, the assessors tasted the beers. Most of the examiners liked the flavor of the beers that contained barley malt. The maize beer was judged at the normal score. The final sensory component was mouth-feel when the beers were evaluated. Mouth-feel is a combination of viscosity, carbonation, astringency, and tactile sensation (whether it is light or heavy on the tongue). Protein, unfermentable sugars (dextrins), beta-glucans, and carbon dioxide influenced a beer's body. According to the results, the assessors liked the beer made from barley while the maize beer was evaluated as acceptable and drinkable.

The overall impression is the description of how favorable each beer was. The maize beer was judged as drinkable and preferred to the next glass while the 2- treatments was determined as good to very good. In conclusion, all experimental beers were acceptable by the assessors. Especially, the beer made from 100% maize malt was evaluated as expected, which indicated that maize malt was a potential material for a new beer product.

Conclusions

The optimal conditions which showed the highest value of malt quality in terms of α - and β - amylases, protease, FAN, Kolbach index, extract content, and reducing sugar were chosen when steeping at 30°C for 24 h and germinated for 5 days at 30°C. The wort obtained from all proportions was suitable for beer fermentation. The sensory test of the maize beer was judged as drinkable and preferred to the next glass. This research proved the viability of maize for brewing technology as a unique product acceptable to the consumer.

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