การผลิตเบียร์ในระดับกึ่งอุตสาหกรรมโดยใช้ข้าวเป็นส่วนประกอบหลัก : การวิเคราะห์สารประกอบที่ระเหยได้และทางประสาทสัมผัส

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บทคัดย่อ

ในการทดลองนี้เป็นการผลิตเบียร์ในระดับกึ่งอุตสาหกรรมขนาด 50 ลิตร โดยใช้อัตราส่วนของข้าวมอลต์และบาร์เลย์มอลต์ที่แตกต่างกัน (50:50, 70:30 และ 90:10) โดยการเติมเอนไซม์ทางการค้าสองชนิด ได้แก่ Termamyl SC ซึ่งเป็นเอนไซม์ α-amylase ที่ทนความร้อนสูง และเอนไซม์ Neutrase ซึ่งเป็นเอนไซม์ protease รวมไปถึงบาร์เลย์มอลต์เพื่อปรับปรุงคุณภาพของน้ำเวิร์ท การทดลองนี้ใช้ข้าวมอลต์ที่ใช้เวลางอก 5 วันในการผลิตเบียร์ ขั้นตอนการบ่มและการอัดก๊าซใช้เวลา 14 วัน และทดสอบคุณภาพของเบียร์ ได้แก่ ปริมาณเอทานอล สี ชนิดและปริมาณน้ำตาลในเบียร์ สารประกอบที่ระเหยได้ และคุณสมบัติทางประสาทสัมผัส ผลการศึกษาพบว่า เบียร์ที่ได้มีปริมาณเอทานอลอยู่ในช่วง 3.18 – 4.00%v/v เบียร์ที่ได้ยังมีปริมาณ isoamyl alcohol อยู่ในช่วง 571 – 620 ppm และ octanoic acid อยู่ในช่วง 364 – 602 ppm ซึ่งมีค่าเกินมาตรฐาน และอาจเกิดกลิ่นไม่พึงประสงค์ในเบียร์ จากผลการทดสอบทางประสาทสัมผัสพบว่า เบียร์ที่มีปริมาณข้าวมอลต์ 50% ให้ค่าความชอบซึ่งอยู่ในเกณฑ์ดี ส่วนเบียร์ที่ใช้ข้าวมอลต์ 70% และ 90% อยู่ในเกณฑ์ที่สามารถดื่มได้

คำสำคัญ: การผลิตเบียร์ในระดับกึ่งอุตสาหกรรม, ข้าวมอลต์, การวิเคราะห์สารประกอบที่ระเหยได้และทางประสาทสัมผัส

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Beer Production from Rice Malt Based in Pilot-scale: Volatile Compounds and Sensorial Properties Analysis

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Abstract

Production of beer from different ratios of rice malt:barley malt (50:50, 70:30 and 90:10) in 50L scale brewing plant was carried out. Commercial enzymes (TermamylSC® as heat-stable α-amylase, and Neutrase® as protease) as well as barley malt were added for improving the quality of rice beer. Fifth day of germinated rice at difference ratios was brewed. Maturation and carbonation steps of beer were performed at 14 days. Qualities of beer from various rice malt ratios including ethanol, color, residual sugar, volatile compounds, and sensorial properties. It was found that, the amount of ethanol in final beer was 3.18 – 4.00 %v/v. Rice malt beer contained isoamyl alcohol of approximately 571 - 620 ppm. The octanoic acid was 364 – 602 ppm which higher than the standard beer might be the off-flavor in beer. The overall impressionable score of 50% rice malt was good, while 70% and 90% rice malt beer were drinkable and can drink more.

Keywords: Pilot-scale brewing, Rice malt, Volatile compounds and sensory analysis

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1. Introduction

Rice (*Oryza sativa*) is the most significant crops in the world as well as wheat and corn, which has been cultivated in over 100 countries around the world. Rice is the essentials food for a half of the world population. The total production of rice paddy area is about 154 million hectares and the annual production of rice is approximately 731 million tons [1]. Thailand is the world class rice production and exporter, which cultivated approximately 32 million tons per year. Nevertheless, rice is inappropriate for brewing due to very high starch content (85-95%). Gelatinizes temperature is 70-85°C due to the presence of very small starch granule. Furthermore, protein content in term of free amino nitrogen (FAN) is low about 5-8% [2] and illustrates the low foam stability and quality. Malt production from Thai rice was studied by Usansa and colleagues [3, 4]. The black rice including Khaw Chao Dam and Khaw Niew Dam could possibly be used as a raw material for gluten free products in the brewing, beverage or food industries. The β-amylase activity of two black rice malts was poorer than barley malt, but had higher amounts of limit-dextrinase and α-glucosidase activity, which could complete starch digestion during mashing and a better attenuation limit. In addition, they had gelatinization temperatures in the medium range (70–75°C), which decreased to less than 70°C after the malting process. The addition of commercial amylolytic and proteolytic enzymes with a less-expensive adjunct has been used. Commercial amylolytic enzymes can be used for increasing fermentable carbohydrates and decreasing viscosity, leading to higher filtration rates and yield, whereas commercial proteases are able to improve the low-molecular weight nitrogenous compounds, especially the free amino acids that play decisive roles in the taste, foam stability and chill sensitivity characteristics of finished beer [5, 6, 7].

The present work aims to study the qualities of rice malt and evaluate the possibility of beer production using rice malt as a major ingredient instead of the commercial barley malt in order to reducing the production cost. Rice malting was carried out and selected the appropriate rice malt for brewing. Then, pilot-scale (50 L) was used for brewing. Finally, chemical properties and sensory evaluation of finished beer were determined.

2. Materials and methods

2.1 Materials

Rice in this research was hybrid medium-short grain rice CP13 obtained from Charoen Pokphand Co. Ltd., Thailand. Barley malt was the *Baudin* species, provided by Khon Kaen Brewery Co. Ltd., Thailand. Brewing enzymes used in this experiment were Termamyl SC® (E.C.3.2.1.1), a heat stable α-amylase that contains 120 KNU/g, and Neutrase® 0.8L (E.C.3.4.24.28) as a protease that contains 0.8 AU/g; both were purchased from Novozymes A/S Denmark. Bottom fermenting yeast was *Saccharomyces cerevisiae* no. 34 from Technical University of Munich (TÜM), Weihenstephan, Germany. Hops pallets was Hopsteiner P90 (4.3% α-acid) purchased from Hopsteiner, Mainburg, Germany.

2.2 Rice malting and analysis of rice malt qualities

Rice malting was carried out using the steeping and air resting programs. The germination temperature and relative humidity were maintained in the incubation chamber at 30°C and 99 %, respectively. Germinated rice was collected on the

1st, 3rd, and 5th days of malting and kilned at 50°C for 24 hours. The rootlets and shoots of dried malt were eliminated. Qualities of the rice malt, including free amino nitrogen (FAN) and extract content, were analysed according to [8] and [9], respectively. The malting loss and the activities of α-amylase and β-amylase were determined according to Usansa et al. [3].

2.3 Beer production

Beer production was done in 50L brewing plant. The 5 days of germinated rice was selected in pilot-scale brewing. Ten kg of malt and 50L of water were mixed. The rice malt and barley malt ratio including 50:50, 70:30 and 90:10 were performed. The mash mixture was done in Mashing tun using the temperature-time profile at 45°C×10 min, 50°C×60 min, 63°C×40 min, and 95°C×60 min. The spent grain was eliminated from wort in the Luater tun.

Then, the wort was boiled at 100°C for 1 hour and hops pellet was added to obtain final beer bitterness for 25 BU during wort boiling. The spent hops and precipitate protein were eliminated in whirlpool tun. The cast wort was moved to fermentation tank and cooled. *S. cerevisiae* was pitched (12×10^6 cell/mL) after cooling and fermented at 14°C for 7 days.

Maturation step at 4°C and carbonation of final beer for 2 week was performed. The brewing diagram was illustrated in Figure 1.

2.4 Chemical analysis

Color of finished beer was performed according to European Brewery Convention, EBC method 8.5, [10]. Residual reducing sugar was analyzed using HPLC (Agilent 1200, Agilent Technology, USA.). Volatile compounds were analyzed using GC-MS (Varian, U.S.A.) by solid phase microextraction (SPME) technique.

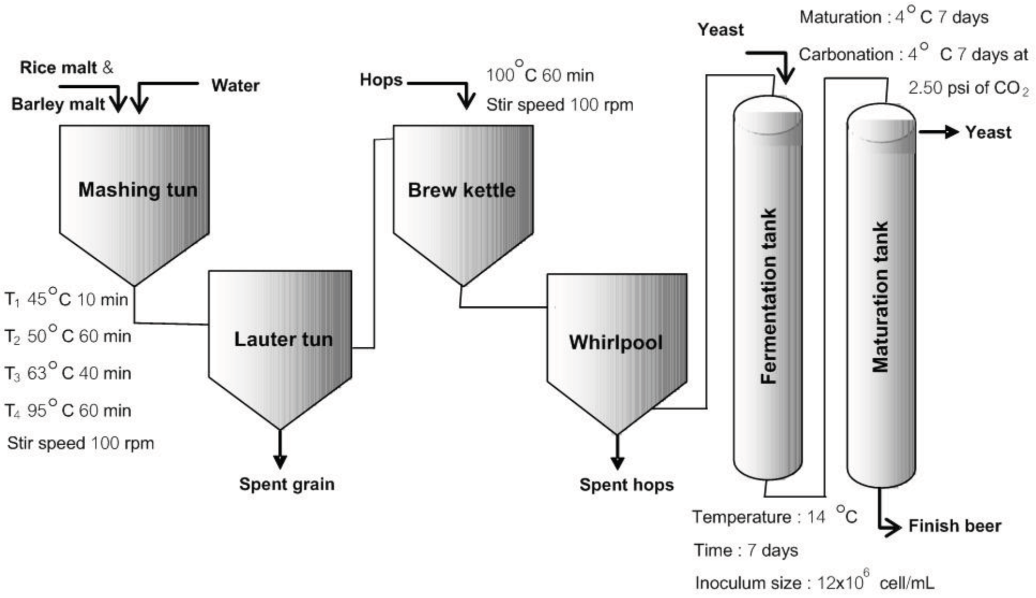

Figure 1 Brewing diagram

2.5 Sensory analysis

The hedonic scale (5 levels) was designed for appearance, aroma, flavor, mouth-feel, and overall-impression. The score of 1, 2, 3, 4, 5 were very dislike, dislike, normal, like, very like, respectively. For overall-impression, the scores 0, 1, 2, 3, 4, and 5 are undrinkable, drinkable but can not drink more, drinkable and can drink more, good, very good, and excellent were analyzed, respectively.

3. Results and discussions

3.1 Rice malt qualities

To investigate the effect of germination time on rice malt quality after 1st, 3rd and 5th day of germination, rice malt qualities were analysed and compared with commercial barley malt, shown in Table 1. The extract content and amylolytic enzymes activities of rice malt were lower than barley malt, especially β-amylase that 5 times lower than barley malt of 5th day germinated rice. The addition of commercial amylase was the suitable way to improve the qualities of wort from rice malt. The malting loss defines as the loss of dry matter that occurred during malting. The result showed that malting loss was corresponding to the germination time due to the loss of storage substance for rootlets and shoots production. The malting loss increased with germination time, up to 17.57%, which is in the normal range for the malting industry. Thus, it is not a main component of operating cost. The FAN in wort was produced from ungerminated rice at 5.22 mg/100 g malt, which is lower than that in malted rice and barley. As a result, the FAN obtained from malted rice increased with germination time. The FAN of 5 days germinated rice was 118 mg/100 g malt which was the closest to the standard range found in barley malt at 120-160 mg/100 g malt [11]. Hence, the 5th day of germinated rice was selected for brewing.

Table 1 Qualities of rice malt from difference germination time compared with control (ungerminated rice) and barley malt

Malt qualities	Control	Germination time (days)			Barley malt
		1	3	5	
Malting loss (%)	-	$3.99^{a}\pm0.727$	$8.78^{b}\pm1.30$	$17.57^{c}\pm1.68$	-
Extract content (%)	$11.26^{a}\pm0.027$	$11.40^{a}\pm0.11$	$15.40^{b}\pm0.187$	$27.36^{c}\pm0.121$	82.1*
FAN (mg/100g malt)	$5.22^{a}\pm0.001$	$18.76^{a}\pm0.388$	$44.70^{b}\pm3.13$	$118.45^{c}\pm4.21$	120-160*
Enzyme activities (Unit)**					
α - amylase	$0.015^{a}\pm0.001$	$0.034^{a}\pm0.0004$	$0.312^{b}\pm0.008$	$0.394^{c}\pm0.006$	$1.052^{d}\pm0.002$
β - amylase	$0.011^{a}\pm0.001$	$0.032^{a}\pm0.0006$	$0.290^{b}\pm0.008$	$0.294^{b}\pm0.025$	$1.489^{c}\pm0.001$

Different letters indicated significant differences in each row (95 % confidence)

* Agu and Palmer (1998)

** Weight of reducing sugars production (g) from starch hydrolysis in 10 min per 1 g of malt

3.2 Chemical properties and volatile compounds of final beer

Chemical properties of final beer including residual reducing sugar, color, and ethanol in beer were analyzed (table 2). The remained maltose was found in the range of 2.59 – 4.63 g/L, while the inefficiency of maltotriose utilization was occurred. The quantity of ethanol in beer was reverse variation with the increasing of rice malt ratio. The maximum ethanol concentration appeared in 50% of malt barley addition at 3.97% (v/v). Color of beer at 50% of rice malt was 20.3 EBC defined as the pale lager beer. The beer color of 70% and 90% rice malt was 30 EBC units, which were in range of standard dark lager beer [12].

Volatile compounds including higher alcohol, estery compounds, and free fatty acid in final beer were determined (table 3). Isoamyl alcohol was in range of 570 – 620 ppm, which was higher than standard beer (60 ppm); and isobutanol was in range of 8.9 – 21.1 ppm, which was in range of standard beer at 9.6 ppm [2]. Isoamyl alcohol is a type of fusel alcohol or higher alcohol that cause of the toxicity in human. Nevertheless, the maximum concentration of isoamyl alcohol which permits in the alcoholic beverage is approximately 3,500 ppm [13]. Higher ratio of rice malt increased the level of isoamyl acetate that might be contributes the banana flavor in beer [14].

The availability of isoamyl alcohol involved isoamyl acetate formation especially in the high gravity fermented wort (18^{O}P) that found the 3 ppm of isoamyl acetate on 120 ppm isoamyl alcohol. Also, addition of 400 ppm of isobutanol increased the isoamyl acetate. Therefore, the isobutanol enhanced the rate of isoamyl acetate production during the fermentation. However, formation of other estery compounds including ethyl acetate, isobutyl acetate, and ethyl hexanoate was unaffected [15].

Table 2 Chemical properties of final beer at difference rice malt ratio after fermentation and aging in pilot scale brewing

Rice malt : Barley malt ratio	Residual sugars in beer (g/L)		Ethanol (%v/v)	Color (EBC)
	Maltose	Maltotriose		
50 : 50	4.63	15.93	3.97±0.05^{c}	20.3±1.01^{a}
70 : 30	3.93	13.29	3.55±0.04^{b}	29.2±1.18^{b}
90 : 10	2.59	12.96	3.18±0.001^{a}	30.5±1.32^{b}

Different letters indicated significant differences in each column (95 % confidence)

Table 3 Volatile compounds of final beer (ppm)

Rice malt : Barley malt ratio	Isoamyl alcohol	Isobutanol	Isoamyl acetate	Ethyl octanoate	Ethyl decanoate	Octanoic acid	Hexanoic acid
50 : 50	585.2^{ab}	17.7^{ab}	18.6^{a}	4.0^{a}	6.9^{a}	364.6^{a}	52.8^{b}
70 : 30	619.1^{b}	21.1^{b}	41.8^{b}	9.9^{b}	13.0^{b}	468.6^{b}	48.1^{a}
90 : 10	571.2^{a}	8.9^{a}	33.6^{b}	10.5^{b}	12.5^{b}	602.4^{c}	54.3^{b}

Different letters indicated significant differences in each column (95 % confidence)

Higher level of rice malt ratio was significantly increase the free-fatty acid in beer especially octanoic acid, that the main cause of the off-flavor in beer due to its contained the high level of fat (approximately 3.0%). On the other hand, free fatty acid improved the formation of estery compounds in final beer. The present of 5 ppm of hexanoic acid and 10 ppm of octanoic acid were characterized as cheesy, goaty, and sweaty flavor [15]. The mechanism of the undesirable flavor in beer is complicacy. However, the number of the off-flavor compounds beside fatty-acid has been reported, for example acetaldehyde, diacetyl, and sulfer compounds [17, 18, 19, 20]

3.3 Sensory evaluation of final beer

Spider diagram of final beer quality aspect was illustrated in Figure 2. It was found that, different level of rice malt had no effect on appearance and aroma of beer. The sensory score of the appearance was 3.0 (like), while aroma was 2.0 (normal), this might due to the remained non-saccharification starch leaded to the opaque beer. Assessors judged as normal flavor and mouth feel in beer at 50% rice malt, but judged as dislike with beer from both 70% and 90% rice malt, due to the sweetness in beer from the remaining sugar by the reason of uncompleted fermentation. Furthermore, panelists judged beer that contained 50% rice malt at 3.0 defined as good, whereas beer from 70% and 90% rice malt at 2.0 defined as drinkable and can drink more. In summary, higher ratio of barley malt addition improved the mouth-feel and flavor of beer, which the main reason of the higher overall impression score, while the quality of beer contained 70% and 90% rice malt was insignificant.

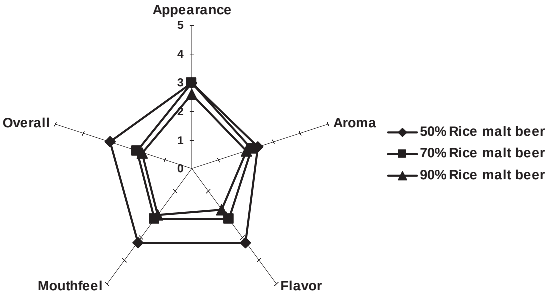

Figure 2 Spider web diagram of the sensory result of beer from rice malt at different ratio

4. Conclusion

The present research is studied the possibility of beer production using rice malt as a major ingredient and applied in pilot-scale. Rice malt at 5 days of germination was the suitable malt for brewing by reason of the closest standard range of FAN. However, extract content was very low. Commercial amylolytic enzymes were added for improved the extract content in wort. The ethanol in beer was in range of 3.18 – 3.97%v/v and color of beer defined as pale and dark lager beer. High level of rice malt improved the formation of estery compounds. On the other hand, the excess of volatile fatty acid and some higher alcohol might be contributed the off-flavor in final beer. For sensory analysis, panelists prefer the 50% rice malt beer than the 70 and 90% rice malt beer that indicated by the over impression score. In addition, the quality of 70 and 90% rice malt beer were insignificant. The sweetness of beer that effect in aftertaste from the existed of non-fermentable sugar, due to the insufficiency of amylolytic enzymes in rice malt.

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