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Effect of mashing process conditions on chemical properties of Kam Luem Pua rice malt syrup

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Abstract

Kam rice malt syrup is an alternative ingredient or raw material in many items in the food and drink industries. The aim of this research was to investigate the optimal conditions for malt syrup production from Thai rice cultivars (*Oryza sativa* L.); black waxy rice (Kam Luem Pua). The mashing program was carried out at three different temperatures (45°, 62°, and 75°C) and times for the sugar rest period with and without the addition of enzyme (T1–T4 mashing method). The results showed that the mashing T3 (at the temperatures 45°, 62° and 75°C, period of 30 minutes and addition of glucoamylase at 60° C for 30 minutes) was suitable to produce high quality wort with a low cost investment. It was found that wort composition had the highest content of reducing sugars (12.07 g/100ml), phenolic compounds (100.56 mg GAE/100ml), anthocyanins (30.45 mg/L), antioxidant activity (76.26%), free amino nitrogen (333.87 mg/L), vitamin B1 (126.53 mg/100ml) and GABA content (4.52 mg/100ml). The results obtained clearly indicated that Kam rice malt has huge potential when used as a raw material for healthy syrup production.

Keywords: *black waxy rice, Kam rice malt, syrup, mashing process, optimal condition, Oryza sativa* L.

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1. Introduction

Rice malt is a product of rice (*Oryza sativa* L. Indica) germinated under control conditions. The germinated grains then undergo processing, such as kilning, grinding, extracting, and filtering, to produce rice malt syrup for the domestic food and beverage industry. During the process of germination, the chemical composition of the rice changes drastically, because the biochemical activity produces essential compounds and energy, for the formation of the seedling (Moongngarm & Saetung, 2010). Apart from changing the levels of nutrients, the biochemical activities which occur during germination, can also generate bioactive components such as ascorbic acid, tocopherols, tocotrienols, γ-aminobutyric acid (GABA), thiamine and phenolic compounds, thus resulting in an increase of antioxidant activity (Komatsuzaki et al., 2007; Trung & Ha, 2016; Vongsudin, Ratthanatham, Laohakunjit, & Kerdchoechuen, 2012). Moreover, it has also been shown to decrease some anti-nutrients, such as phytic acid, which interfere with the absorption of important vitamins and minerals (Watchararpapaiboon, Laohakunjit, & Kerdchoechurn, 2010). Germinated Thai rice has rarely been used in the beverage industry. Recently, it has been reported that two black rice varieties, black non-waxy rice and black waxy rice malts may show promise in this area. The results obtained from this research clearly demonstrate that black rice malt could be used as a raw material for producing beers with an acceptable quality and suggest that rice malt has potential for use in brewing (Teaumroong, Boonkerd, & Wanapu, 2010). Further, germinated brown rice is already being used to produce beverage products (Wongpiyachon, Sudtasarn, & Sukviwat, 2011). Thus, the use of black rice malt as a major raw material for syrup production in the beverage industry would be a new challenge.

Mashing is the most important process in wort production. During mashing, the rice malt grist and water are brought into suspension. During this step, sugars are extracted and the nutrients and enzymes are then allowed to react at their optimum temperatures and times. Only soluble substances can pass into the syrup. The aim of mashing is to form as much extract and as good an extract as possible (Kunze, 2010). Many factors need to be considered when mashing: different types, different mashing temperature programs, use of enzymes to increase yield (Goode, Halbert, & Arendt, 2003; Goode, Wijngaard, & Arendt, 2005) and pH adjustments (Bamforth, 2001). Moreover, there are many reports of improving the mashing procedures to improve extract yield, wort quality and lowering investment costs (Alvarez, Correa, Navaza, & Riverol, 2000; Goode et al., 2005; Igyor, Ogbonna, & Palmer, 2001; Kongkaew et al., 2012). The addition of alpha-amylase and glucoamylase was applied in the mashing process but there are no reports that the enzymatic mashing process has been optimized to produce rice malt syrup making in Thailand. Further, because these enzymes are expensive their applicability is questionable in this process.

2. Objectives

The aim of this study is to optimize the effects of 4 mashing methods by using different temperatures and enzymes to increase the extract yield for reducing sugar production in 2 hours, increase wort quality and lower investment costs on the final rice malt syrup quality from black waxy rice (Kam).

3. Materials and methods

3.1 Raw material

Rice malt was made from sprouted paddy rice (*Oryza sativa* L.); black waxy rice (Kam Luem Pua). Kam Luem Pua was purchased from the local farming at Kheknoi Village, Khaokor District, Phetchabun Province, Thailand. Manufacturing time was November 2015. Commercial α-amylase (30,000 unit) and glucoamylase (100,000 unit) enzymes were purchased from ONIMAX Co., Ltd., Bangkok, Thailand and stored at 4°C.

3.2 Malt preparation

Cleaned Kam Luem Pua rice of paddy rice was steeped in tap water at room temperature (30°C) and the water was changed every 12 hours and drained for 1 hour until the moisture content was constant. The germination took place in a germinating incubator for 72 hours, at 30°C and 95% relative humidity. The germinated seeds were kilned at 70°C for 9 hours and the acrospire and rootlet were removed by following the method of Nalad and Puangwerakul (2014).

3.3 Grist preparation

Rice malt was milled by a pin mill to give fine particle sizes of 250-micron grist.

3.4 Wort preparation

The grist was mashed with distilled water at the ratio of 1:4 w/w and operated with a wort temperature-program. The temperature-program of wort for mashing of rice malt was produced by following a modified procedure of wort preparation for beer production. Rice malt grits, 70 g, were mixed with distilled water, 280 ml, for 2 hours, the spent grain was then removed from the wort after the mashing process. Four mashing methods were performed as seen below (Table 1).

Table 1 The four mashing methods used for wort production

Treatment 1(T1)	Treatment 2(T2)	Treatment 3(T3)	Treatment 4(T4)
45, 62 and 75 °C for 40 min	45, 62 and 75 °C for 30 min	45, 62 and 75 °C for 30 min	amylase 0.28 ml, pH 5.8-6.5, 75 °C for 1 hour
	alpha-amylase 0.28 ml, pH 5.8-6.5, 75 °C for 30 min	glucoamylase 0.28 ml, pH 4-4.5, 60 °C for 30 min	glucoamylase 0.28 ml, pH 4-4.5, 60 °C for 1 hour

3.5 Filtration

The wort was filtered using a kieselguhr as a filter aid.

3.6 Syrup analysis

The amount of reducing sugar was determined using the 3,5-dinitrosalicylic acid (DNS) method (Miller, 1959). The pH value of wort and syrup was determined by pH-meter. The antioxidant activity was determined by the DPPH radical scavenging method (Brand-Williams, Cuvelier, & Berset, 1995). Total phenolic compounds were determined by the Folin-Ciocalteau phenol test (Singleton & Rossi, 1965). Vitamin B1 was determined according to the method of Liu, Zhang, Liu and Luo (2002). GABA was determined by the modified procedure of Kitaoka and Nakano (1969). Free Amino Nitrogen (FAN) was determined by the method of European Brewery Convention (EBC) (1987). Anthocyanin was determined by the pH-differential method (Lee, Durst, & Wrolstad, 2005).

3.7 Statistical analysis

Results are expressed as the mean values and 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 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

During mashing, the grist is dissolved, and the insoluble residues are separated from the spent grains. The major part of the extract produced in mashing consists of extracted sugars and nutrients. Only soluble substances can pass into the syrup (Kunze, 2010). The extraction yield (in %) of wort from Kam Luem Pua rice malt prepared by four mashing methods was not significantly different (70.71-75.00%). In this work, it was found that the amount of reducing sugars in the extracts was significantly higher (13.78 and 12.07g/100ml) in T4 and T3 than in T2 and T1 (7.30 and 6.53 g/100ml). The wort produced from mashing T1 modified from beer making by proteinase activity was performed at 45°C. FAN and soluble nitrogen were determined as products of an enzymatic reaction (Kunze, 2010). The β-amylase was activated at 62°C for maltose production and α-amylase was activated at 75°C for glucose production (Puangwerakul, Lekpan, & Khamhaeng, 2014). Then, mashing T2 was extracted by following the T1 process and the commercial enzyme α-amylase was added, which hydrolyzes starches to dextrins by cleaving the random α-1→4 linkages in an endo-manner and non-reducing end resulting in mostly dextrins and some glucose (Johnson, Padmaja, & Moorthy, 2009). While the T3 mashing was extracted by following process T1 and the addition of the commercial enzyme glucoamylase, the enzyme glucoamylase hydrolyzes the dextrins to glucose by attacking both α-1→4 and α-1→6 linkages at the non-reducing ends (Labout, 1985). Additionally, mashing T4 was extracted by using two commercial enzymes, α-amylase and glucoamylase, yielding the highest content of reducing sugar. However, the qualities of reducing sugar from T3 and T4 were not significantly different.

The vitamin B1 content in T4, T3, T2 and T1 were 156.94, 126.53, 108.83 and 87.39 mg/100ml, respectively. The results showed that the mashing T4 had highest content of vitamin B1 in the range of pH 5.8-6.5 and 75°C for 1 hour. The results were similar to Puangwerakul et al. (2014), who reported vitamin B1 content in wort mashing at 75°C was higher than mashing at 65°C. Moreover, the pH conditions were similar to the conditions of the enzyme thiamine phosphate synthase (EC 2.5.1.3) and enzyme thiamin diphosphate kinase (EC 2.7.6.2). The optimal conditions for these enzymes were between pH 7-7.5 (Yamada & Kawasaki, 1980). Tanaka, Sugimoto, Ogawa, and Kasai (1980) 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 and cause the change in thiamine levels. Therefore, the dissolved free thiamine content is higher. This mechanism occurs in wheat seeds during germination (Watanabe et al., 2004). The mashing T2 had the highest content of GABA (6.01 mg/100 ml), followed by the mashing of T3, T4 and T1 (4.52, 3.58 and 3.23mg/100ml, respectively). In the adjusted pH conditions of slightly acidic, H^+ stimulated the activation of the enzyme GAD (glutamate decarboxylase) changing glutamic acid to GABA. Therefore, the amount of GABA increased (Watchraparpaiboon, Laohakunjit, Kerdchoechuen, & Photchanachai, 2007).

The total phenolic compounds, anthocyanin, and antioxidant activity in the four mashing processes of wort were similar (100.22-112.45 mgGAE/100 ml, 29.07-36.11 mg/L, 76.26-81.31 % Scavenging effect, respectively). Ruenroengklin et al. (2008) reported that phenolic compounds increased when a higher temperature for extraction of phenolics was used. Randhir and Shetty (2005) indicated that the antioxidant activity was linked to free soluble phenolics and anthocyanins (Ming-weil et al., 2006). Anthocyanins, a group of reddish to purple water soluble flavonoids, are the primary pigments in the red and black grains, and are widely identified and characterized in cereal grains (Abdel-Aal, Young, & Rabalski, 2006). The major components of anthocyanidins in colored rice are cyanidin-3-glucoside and peonidin-3-glucoside (Abdel-Aal et al., 2006; Yawadio, Tanimori, & Morita, 2007). 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 increase of antioxidant efficiency and dissolvability of wort from mashing might be due to the changing of antioxidant structure at higher temperatures.

Table 2 Characteristics of Kam Luem Pua rice malt syrup

Parameter (Unit)	Mashing method			
	T1	T2	T3	T4
Reducing Sugar (g/100ml)	6.53±1.15^{b}	7.30±0.98^{b}	12.07±1.27^{a}	13.78±3.39^{a}
Vitamin B1 (mg/100ml)	87.39±3.48^{d}	108.83±3.33^{c}	126.53±9.14^{b}	156.94±5.76^{a}
GABA (mg/100ml)	3.23±0.27^{c}	6.01±1.13^{a}	4.52±0.60^{b}	3.58±0.26^{c}
TPC (mg GAE/100ml)	100.22±14.17^{a}	109.80±14.38^{a}	100.56±8.56^{a}	112.45±11.75^{a}
Anthocyanin (mg/L)	36.11±5.65^{a}	29.07±3.38^{a}	30.45±4.17^{a}	30.23±7.15^{a}
Scavenging effect (%)	76.62±9.10^{a}	81.31±3.31^{a}	76.26±10.26^{a}	78.91±13.27^{a}
FAN (mg/L)	281.53±15.23^{b}	270.19±25.38^{b}	333.87±56.45^{a}	344.35±4.45^{a}
pH	5.49±0.18^{a}	5.63±0.34^{a}	5.05±0.09^{b}	4.86±0.31^{b}
%Yield	70.71±8.20^{ns}	71.78±7.14^{ns}	72.59±2.91^{ns}	75.00±7.75^{ns}

Values represent mean ± standard deviation of means.
Mean values within the same row sharing the same superscript were not significantly different at $p \leq 0.05$.
FAN = Free Amino Nitrogen, TPC = Total Phenolic Compound, Scavenging effect was determined by the procedure of Brand-Williams et al., 1995.
^{ns} no significant difference.

For consideration of FAN content, it was indicated that the action of proteases, breaking down protein to soluble free amino nitrogen, was significantly higher (344.35 and 333.87 mg/L) in T4 and T3 than in T2 and T1 (270.19 and 281.53mg/L). The optimum temperature range for the proteinase 45-50°C at less than pH 5.3 (Kunze, 2010). The results showed that the mashing T4 had highest amount of vitamin B1, but reducing sugar, total phenolic compound, free amino nitrogen, anthocyanin and antioxidant activity were not significantly different in the mashing T3. The mashing T3 had a higher content of GABA than the mashing T4. This may be due to the use of two enzymes in the mashing T4 process, which is a cost-intensive process because of the enzyme steps. The cost of mashing T3 could be reduced by using only one enzyme. The chemical properties of syrup from mashing T3 and T4 were only slightly different despite the additional enzymes. Therefore, the mashing T3 was the optimal condition, which had the highest content of reducing sugar, phenolic compound, anthocyanin, antioxidant activity, and free amino nitrogen, 12.07 g/100ml, 100.56 mg GAE/100ml, 30.45 mg/L, 76.26%, and 333.87 mg/L, respectively. Vitamin B1 and GABA contents were 126.53 mg/100ml and 4.52 mg/100ml, respectively.

5. Conclusion

Kam Luem Pua rice malt syrup had the highest qualities of wort obtained from the mashing T4, but this mashing process used two enzymes, which is a cost-intensive step. The cost of mashing T3 could be reduced by using only one enzyme. The chemical properties of wort from mashing T3 and T4 were slightly different. Therefore, the mashing T3 shows the optimal condition of wort quality with the lowest investment costs. The main advantage of using Kam Luem Pua rice malt as raw material for syrup production is not only from its source of enzymes, but also its source of bio-functional compounds that can add value to Thai rice. This data will be used for a further related study and will be applied to the domestic food and beverage industries as an alternative healthy source of raw materials.

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