

Effect of soaking, germination and roasting on thiamine content in Pathum Thani 1 rice malt

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Abstract

Malting is the three step-process for achieving high quality and nutritious cooking rice with specific characteristics and flavor. Considerations for time and temperature are important in efficiently achieving the highest nutritional content. This process effectively increases the smaller peptide and oligosaccharide contents, especially amino acids and reducing sugars, which results in the development of color, taste, and aroma in malt. Experiments found that thiamine, a surrogate for overall vitamin content increased significantly by soaking in water peaking at 1.3 times unsoaked rice levels when soaked for 60 hours (0.237 and 0.185 mg/100 g, respectively). Moreover, it was found that period of germination greatly affects thiamine content. With extended germination periods, thiamine progressively increased from 0.237 mg/100 g to 0.758 mg/100 g (3.2 times) when paddy germinated for 72 hours. Malted rice was roasted at 160, 170, 180, 200, and 250°C for 20, 40, and 60 minutes in a drum rotary roasting machine. It was found that higher temperature and extended time led to significant reduction of thiamine content. However, for the industrial-scale production, the rice should be roasted at 170°C for 20 minutes in order to maintain both desired thiamine content and physical properties, such as color and flavor, corresponding with desired malt appearances. Consequently, these conditions could maintain sufficient thiamine content at 0.293 mg/100 g, which meets the level of 20% Thai RDI criterion.

Keywords: soaking, germination, roasting, malting, thiamine

บทคัดย่อ

กระบวนการมอลต์ เป็นวิธีการแปรรูปที่ได้พัฒนาขึ้นสำหรับใช้ผลิตข้าวหุงรับประทานคุณภาพสูงทางโภชนาการ ที่มีความพิเศษในลักษณะปรากฏและกลิ่นรส เป็นกระบวนการที่ช่วยเพิ่มปริมาณเปปไทด์ขนาดเล็กและโอลิโกแซคคาไรด์ โดยเฉพาะอย่างยิ่ง กรดอะมิโนและน้ำตาลรีดิวซ์ ซึ่งมีผลต่อการพัฒนาของสี รสชาติ และกลิ่นหอมในมอลต์ ผลการทดลองพบว่าปริมาณไทอามีนเพิ่มขึ้นอย่างมีนัยสำคัญในขั้นตอนการแช่น้ำ การเปลี่ยนแปลงในปริมาณไทอามีนในระยะ 60 ชั่วโมงของการแช่น้ำเพิ่มขึ้นเป็น 1.3 เท่าเมื่อเปรียบเทียบกับเมล็ดที่ไม่ได้แช่ (0.237 และ 0.185 มิลลิกรัม/100กรัม ตามลำดับ) ยิ่งไปกว่านั้น พบว่าขั้นตอนการงอกมีผลต่อปริมาณไทอามีนอย่างมาก โดยเมื่อเพิ่มระยะเวลาในการงอกให้ข้าวออกไป ปริมาณไทอามีนมีการเพิ่มจาก 0.237 มิลลิกรัม/100 กรัม เป็น 0.758 มิลลิกรัม/100 กรัม (3.2 เท่า) ในการเพาะข้าวเปลือกเป็นเวลา 72 ชั่วโมง ข้าวมอลต์ ถูกนำมาคั่วที่อุณหภูมิ 160 170 180 200 และ 250 องศาเซลเซียสด้วยเครื่องคั่วแบบลูกกลิ้ง โดยแปรเวลาในแต่ละระดับอุณหภูมิที่ 20 40 และ 60 นาที พบว่าการใช้อุณหภูมิสูงและระยะเวลาที่นานขึ้น ทำให้ปริมาณไทอามีนลดลงอย่างมีนัยสำคัญ ได้คัดเลือกอุณหภูมิการคั่วที่ 170 องศาเซลเซียสเป็นเวลา 20 นาที สำหรับการผลิตระดับอุตสาหกรรม เพราะมีผลทำให้ได้ทั้งปริมาณไทอามีนและสมบัติทางกายภาพ ได้แก่ สี และกลิ่นรส ที่ตรงกับลักษณะปรากฏที่ดีของมอลต์ที่ต้องการ และที่สภาวะดังกล่าว ยังคงมีปริมาณไทอามีนที่น่าพอใจ เท่ากับ 0.293 มิลลิกรัม/100กรัม ซึ่งอยู่ในระดับร้อยละ 20 ของเกณฑ์มาตรฐาน Thai RDI

คำสำคัญ: การแช่, การงอก, การคั่ว, มอลต์, ไทอามีน

1. Introduction

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, 2003). Many components of the vitamin B complex exist in abundance compared with other vitamins. Vitamin B found in rice is comprised of thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), pyridoxine (B6), and

folic acid (B9), which are all highly soluble in water. B vitamins in rice and other cereal grains are concentrated in the aleurone layer and in the embryo (Juliano, 1985; Batifoulier, Verny, Chanliaud, Remessy, & Demigne, 2006). Given the many deficiency diseases associated with a lack of vitamin B complex vitamins, the high concentrations of B vitamins seen in rice underscore its importance in diet and disease prevention.

Malted rice products for cooking and consumption adapt the malting process for Indica rice. The malting process for rice malt production consists of three important stages: steeping, germination and roasting. During steeping, the grain absorbs the water necessary for germination and is then germinated in large tanks. Germination is the stage that produces the enzymes of hydrolysis and modifies the storage starch and protein in the rice kernels. Germination is terminated by roasting at a high temperature, which affects the properties of rice grains in three major ways, which are color, aroma, and taste. These properties are completely different from original and germinated brown rice (Puangwerakul, 2010; Puangwerakul, 2011). It was found that the enhanced sweet flavor is a result of increased total sugar content. The sweet aroma, called malty flavor, is derived from compounds in the methyl alcohol group converted from degradation of amino acids (Sheldon, Lindsay, Libbey, & Morgan, 1971). The dark reddish-brown color is the result of both a maillard reaction between amino acid and reducing sugar when heated and from a caramelization reaction of burnt sugar in the rice roasting stage (Wolfgang, 1999).

Thiamine functions as a co-enzyme that is crucial for respiratory processes, degradation of carbohydrates, and growth of cells in germinating seeds. Examples of these enzymes are pyruvate dehydrogenase, α -ketoglutarate dehydrogenase, transketolase and phosphoketolase. Thiamine functions as both catalyst and reaction originator. To remain healthy and continue propagation, cells need to synthesize this in sufficient quantities (Gregory, 1996). In this regard, Belanger, Leustek, Chu, and Kriz (1995) explained that thiamine is an essential constituent of all cells. In cereal grains, thiamine is concentrated in the aleurone layer and in the embryo (Batifoulier et al., 2006). Including the husk in the germination process can enhance vitamin B content which, aside from not being lost in the soaking stage, results in enhanced vitamin B content. Briggs (1998) summarized the change of vitamin Bs in malt that thiamine and niacin are altered very little while riboflavin, pantothenic acid and pyridoxine increased during germination. The malt vitamins carried forward into beer may have nutritional significance for consumers. The goal of this research project is to monitor thiamine in all process stages as thiamine (B1) can be an excellent substitute for other B vitamins. There is a strong correlation between the concentration of thiamine and other B complex

vitamins. Because of this, it can be used to gauge the overall changes in all B vitamins during the malting process. A previous study has shown the increase in all forms of B vitamin in malt, germinated barley, that thiamine content increased from 1.2 to 2.4 $\mu\text{g/g}$, riboflavin increased from 0.8-3.7 to 1.2-5.0 $\mu\text{g/g}$, niacin increased from 47-147 to 48-150 $\mu\text{g/g}$, pantothenic acid increased from 2.9-11.0 to 4.3-12.9 $\mu\text{g/g}$, pyridoxine increased from 2.7-11.5 to 3.8-7.5 $\mu\text{g/g}$, and folic acid increased from 0.2 to 0.4 $\mu\text{g/g}$ (Brigg, 1998; Briggs, Hough, Stevens, & Young, 1981). Puangwerakul (2010) reported changes in thiamine content in 15 varieties of Thai rice during germination that had a tendency to increase throughout the period of germination at an average rate of 3.4 times. This is consistent with research by Kayahara and Tsukahara (2000) that found an increase in thiamine content in pre-germinated brown rice that exceeds white rice by a factor of 3.

While thiamine can be used to assess the overall levels of B vitamins, it is important to note that thiamine is sensitive to heat degradation. The first two stages of the malting process, steeping and germination, help increase thiamine content. However it is currently unknown what occurs to these levels during the process of roasting at temperatures of 160-250°C.

Therefore, this study seeks to empirically determine the appropriate time periods and temperature levels for commercial production of rice, while retaining properties of color, aroma, and flavor specific to characteristics of malted rice. The fulfillment of this study can be applied to other cultivars of Thai rice.

2. Objectives

This study was aimed to determine the effect of soaking, germination, and roasting on proximate thiamine content in Pathum Thani 1 rice malt.

3. Materials and methods

3.1 Raw material

Pathum Thani 1 paddy rice was obtained from rice fields at Sahn Khok District, Pathum Thani Province and harvested in November 2008. Paddy rice was kept for 4 weeks until past the seed dormancy period with final moisture content of 12%.

3.2 Malt preparation

3.2.1 Soaking

Pathum Thani 1 paddy rice was soaked in water, pH 7.0 in a tank with a capacity of 125 Kg/m². Temperature was kept constant at 30°C and air provided at a rate of 50 litre/m²/min for 0-84 hours. Samples were collected every 12 hours.

3.2.2 Germination

Seeds were cultivated to germinate in the dark. Relative humidity was controlled at 90% for 0-120 hours. Samples were collected every 12 hours.

3.2.3 Roasting

Green malt was roasted in a temperature-controlled rotary drum roasting machine, by varying roasting temperatures at 5 levels: 160, 170, 180, 200, and 250°C for 60 minutes and varying times used to roast in 3 phases: 20, 40, and 60 minutes.

3.3 Sample preparation

The samples were kilned by tray dryer at 55°C to reduce final humidity to 10% and polished at 70% level using a rice huller. Rice grains were ground to powder and stored in vacuum-sealed foil bags at 4°C.

3.4 Moisture content analysis

The moisture content was determined from the loss in weight of 5 g of ground sample after heating for 3 h at 105°C by the method of European Brewing Convention (EBC, 1987).

3.5 Diastatic power analysis

Diastatic power analysis was done by the method of EBC (1987). Twenty grams of rice malt powder were placed into the mash beakers. The mashing bath was heated to 40°C, with, four hundred and eighty milliliters of distilled water added and stirred with a glass rod to avoid balling. Malt enzymes were extracted with distilled water at 40°C for 1 h. The extraction solution was cooled to room temperature and the contents of the beaker adjusted to 520 g. One hundred milliliters of standard starch solution was hydrolyzed by the malt enzyme extract by pipetting into 200 ml volumetric flask. Five milliliters of Acetate buffer was added and placed in water bath at 20°C for 20 min. Five milliliters of malt extract were added and the contents of the flask shaken thoroughly. The solution was left in the 20°C water bath for 30 min. Four milliliters of sodium hydroxide was added to inactivate the enzymes. Total volume was made up to 200 ml with

distilled water and mixed well. The result was calculated as grams of maltose which was produced under the specified conditions by 100 g of malt.

3.6 Germinative energy analysis

Germinative Energy (GE) analysis was done by the method of EBC (1987). Filter papers, Whatman No.1, were placed in the bottom of the Petri dish and 4 ml of water was added. One hundred grains of paddy rice were placed on the paper so that each grain made good contact with the paper. The dish was covered with its lid ensuring a good seal and to prevent evaporation. All the dishes were placed in a sealed polyethylene bag and incubated in a dark cabinet at 30°C for 72 h. The non-germinated grains were counted. A grain was considered as germinated if rootlets or the acrospires were visible with the naked eye. The percentage of germination was calculated as the Germinative Energy (% GE).

3.7 Thiamine analysis

Thiamine analysis was performed as previously described by Liu, Zhang, Liu, Luo and Zheng (2002). One gram rice malt powder was placed in 100 ml calibrated flask. The sample was prepared by adding 1.5 ml NH₄Cl-NH₃.H₂O buffer (49 ml of 0.2 mol/L NH₄Cl and 1 ml of 0.2 mol/L NH₃.H₂O, pH 7.6) and solubilization agent (1%) 1.0 ml Triton X-100 for phenol red (PR). The mixture was diluted to 15 ml with water. Dye solution (0.05%) 1.5 ml for PR was added and the mixture was diluted to 100 ml with water. The solution was incubated for 10 min at RT for PR. Absorbance was measured at 427 nm using a spectrophotometer. A standard stock solution containing 500 µg/ml of vitamin B1 was prepared by dissolving 0.025 g of thiamine in deionized water. The standard concentrations of 10, 20, 30 and 40 µg/ml were prepared from the stock solution. All determinations were carried out in triplicate.

3.8 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 determined as significant by Duncan's new multiple range test (DMRT) when $p < 0.05$. All analyses were carried using the SPSS program.

4. Results and discussions

4.1 Effect of soaking time on thiamine content of rice

Production of Pathum Thani 1 malted rice in the soaking stage was found that rice paddy absorbed greater amounts of humidity into the grain until saturation or constant moisture content was at 28% at the 60 hour (Table 1). Moreover, when soaked further, moisture content in the rice grain no

longer rises and at this level of moisture content in the 60 hour of soaking can stimulate the highest rate of cultivation at the standard germinative energy level of 96% according to the criterion of pale malt set by the EBC (1987), which specified that the criterion must exceed or equal 95% and yet still affect increase of thiamine content from base level in rice paddy 0.185mg/100 g to 0.237 mg/100 g, as shown in Table 1.

Table 1 Effect of soaking time on moisture content, germinative energy and thiamine content in Pathum Thani 1 paddy rice

Soaking time (Hour)	Moisture content (%)	Germinative energy (%)	Thiamine content (mg/100 g)
0	12±0.8 ^E	0	0.185±0.002 ^E
12	13±0.6 ^E	27±5.4 ^D	0.200±0.002 ^{DE}
24	17±0.5 ^D	65±5.4 ^C	0.204±0.004 ^{CD}
36	20±0.5 ^C	78±6.4 ^B	0.216±0.008 ^{BCD}
48	22±0.4 ^B	90±5.5 ^A	0.230±0.008 ^{AB}
60	28±1.0 ^A	96±5.0 ^A	0.237±0.010 ^A
72	28±0.6 ^A	95±5.5 ^A	0.220±0.010 ^{ABC}
84	28±0.2 ^A	95±4.5 ^A	0.221±0.018 ^{ABC}

The mean comparison in the same column with different letters were significantly different at $p \leq 0.05$.

From Table 1, a significant trend of thiamine content increase was found from the start at zero hours and peaking at the level of 0.237 mg/100 g at the 60 hour of soaking, an increase of 1.3times. This is consistent with research conducted on wheat by Lemar and Swanson (1976), research conducted on millet by Opuku, Ohenhen and Ejiofor (1981), research conducted on cereals by Chavan and Kadam (1989), and research conducted on Thai rice by Watcharparpaiboon, Laohakunjit, and Kerchoechurn (2010) conducted on Thai rice. After 60 hours, thiamine content tended to be constant and not increase further. This is due to pH of soaking water that changed from pH 7.0 from the dissolving of CO₂ from cell activity making soaking water more acidic (pH about <6.0) coupled with heat from seed growth activity. Consequently, this is not suitable for respiratory processes and thiamine synthesis because the activity of two types of enzymes that function to synthesize thiamine: thiamine phosphate synthase (EC2.5.1.3) and thiamine diphosphate kinase (EC2.7.6.2), function well at pH 6.0-7.0 and 30-37°C (Yamada & Kawasaki, 1980). This was in agreement with earlier report. It was found that some plants and bacteria can synthesize thiamine via a similar pathway (Rapala-Kolik, Kowalska, & Ostrowska, 2008).

4.2 Effect of germination time on thiamine content of rice

Monitoring thiamine content during germination at 0-120 hour, analysis was done concurrently with the value of diastase enzyme activity, as shown in Table 2.

Table 2 Effect of germination time on diastatic power and thiamine content in Pathum Thani 1 malt

Germination time (Hour)	Diastatic power (WK-unit)	Thiamine content (mg/100g)
0	4.0±0.5 ^H	0.237±0.033 ^F
12	4.0±0.2 ^H	0.359±0.040 ^E
24	24±1.2 ^G	0.472±0.031 ^D
36	66±1.0 ^F	0.576±0.037 ^C
48	74±1.2 ^E	0.631±0.028 ^C
60	115±1.0 ^D	0.706±0.045 ^B
72	122±1.2 ^A	0.758±0.031 ^{AB}
84	119±1.2 ^B	0.800±0.040 ^A
96	119±1.0 ^B	0.801±0.042 ^A
108	117±1.2 ^C	0.801±0.035 ^A
120	114±1.5 ^D	0.802±0.040 ^A

The mean comparison in the same column with different letters were significantly different at $p \leq 0.05$.

From Table 2, it was found that when the germination period was extended, thiamine content tended to increase from start (0.237 mg/100 g) and peak at 72 hours of germination (0.758 mg/100 g), or 3.2 times the starting concentration. After 72 hours, the thiamine content increased slightly to 0.800, 0.801 and 0.802 mg/100g but it was not statistically significant. The results elucidated 72

hours was the shortest germination time and was good for cost saving also. This is consistent with results reported by Zhang, Hu, Tang, Zhao, and Wu (2005) conducted on germinated brown rice which found that germination can increase thiamine content by 2.5 times. Additionally, Briggs et al. (1981) found that germinated barley can increase thiamine content up to 2 times and thereafter begin to level out and tending to gradually decrease respectively. Furthermore, it is noticeable that the increase in thiamine content is consistent with the increase of diastase content.

Amylase, which is an enzyme that has an important role in digesting starch in rice paddies, is produced while the grain is beginning to germinate (post soaking period) and developed simultaneously with grain germination. Thus, it follows that when germination time is extended diastase content increases also. Juliano (1985) described that the activity of amylase increased progressively during starch degradation by four days of germination. Glucose was the major product of starch degradation. Sucrose, maltose, maltotriose, raffinose, and fructose were also detected (Kiribuchi & Nakamura, 1974). Moreover, sugar content depended on the functional activity value of the enzyme. In this regard, the change in profile of diastase content during germination will gradually increase until the peak concentration followed by a sustained drop. It is note worthy that the increase in diastase content occurs during a similar period with the increase in thiamine content. This is because theoretically thiamine is synthesized to function as a co-enzyme in an activity that digests large molecules, especially starch into smaller molecules. Thus, it supports the reasoning that the period that finds the greatest

increase of enzymes will be similar to that of increase in thiamine content. This is because during germination there is an accumulation of vitamins in preparation for photosynthesis during creation of green leaves (chlorophyll formation). The increase in diastase content is consistent with research reported by Puangwerakul (2007), who found that the greatest increase in malt prepared from Thai rice was at a germination period from 48 hours and beyond and reported that most rice varieties have diastase values between 52-159 WK-unit, which are lower than wheat and barley malt (166-184 WK-unit), and standard malt, according to malt criteria of EBC (1987).

For levels of thiamine, suitable germination periods can be chosen from the 12 hour, which provides levels greater than 0.3 mg/100g. Because criteria announced by the Ministry of Public Health (1995) in 1995 citing thiamine content in products per unit consumption as “High” or “Enriched” must contain no less than 20 percent of the Recommended Daily Intake (RDI) value for Thai people from 6 up (Thai RDI). However, rice grains are still not sweet as required. Therefore, in deciding a suitable germination period, besides considering vitamin content as “High” or “Enriched,” it must be the period that also shows high diastase enzyme content. It can be said that the 72 hours of germination is the best period, rice will have a sweet taste and thiamine increasing rate is at 3.2 times, or equivalent to 50% of Thai RDI.

4.3 Effect of roasting temperature and time on thiamine content of rice

Experiments showing the results of roasting to changes in thiamine content are shown in Table 3.

Table 3 Effect of roasting temperature and time on thiamine content in Pathum Thani 1 malt

Roasting temperature (°C)	Thiamine content (mg/100g) after roasting time (min)			
	0	20	40	60
160	0.760±0.016 ^{Aa}	0.392±0.015 ^{Ab}	0.310±0.012 ^{Ac}	0.171±0.015 ^{Ad}
170	0.762±0.015 ^{Aa}	0.293±0.021 ^{Bb}	0.274±0.021 ^{Bb}	0.151±0.017 ^{ABc}
180	0.761±0.015 ^{Aa}	0.288±0.014 ^{Bb}	0.237±0.012 ^{Cc}	0.151±0.012 ^{ABd}
200	0.762±0.018 ^{Aa}	0.235±0.022 ^{Cb}	0.183±0.012 ^{Dc}	0.141±0.012 ^{BCd}
250	0.761±0.015 ^{Aa}	0.178±0.014 ^{Db}	0.138±0.010 ^{Ec}	0.134±0.012 ^{Bc}

The mean comparison in the same column with different capital letters and that in the same row with different minuscule letters were significantly different at $p \leq 0.05$.

From Table 3, it was found that higher temperatures and longer time significantly reduced thiamine content because the thiamine structure was unstable and more heat labile. Moreover, reduction in thiamine also occurred from maillard reactions between carbonyl group from sugar and amine from amino, peptide, protein, and thiamine. Sapers (1993) provided an explanation of nonenzymatic browning of maillard reaction type that the reaction rate would increase 2-3 times when temperatures increased every 10 °C. This reasoning supported experimental outcomes that found loss of thiamine content in greater rates when rice roasting temperatures and time were increased. Nevertheless, in selecting temperature levels to use in roasting, the most important issue was color development of rice grains. It was found that temperature levels that return good results in color were temperatures that were higher or equivalent to 170°C or 160°C in excess of 30 minutes (color data not shown). Therefore, if thiamine levels were referenced as “High” or “Enriched” at 0.3 mg/100 g, this was the selection criteria of the most suitable temperature and time for roasting malted rice. One could select 160°C for 40 minutes (thiamine remaining 0.310 mg/100g) and 170°C and 180°C for 20 minutes (thiamine remaining 0.293 and 0.288 mg/100 g, respectively). Nonetheless, it was found that roasting at 160°C took up to twice longer than those at 170°C and 180°C. Thus, when considering suitability relating to industrial labor and production cost expenses, 170°C for 20 minutes was preferred. Malted rice grains undergoing roasting at this temperature showed a darker hue of brown than those roasted at 160°C. This was consistent with theory of color change from caramelization reaction that required temperature as high as 170°C to get caramel color and malt aroma (Wolfgang, 1999).

5. Conclusion

Changes in thiamine content in producing Pathum Thani 1 malted rice were found to increase compared to basic brown rice when periods of soaking and germination were extended. In this regard, the appropriate soaking period was 60 hours, which increased thiamine content up to 1.3 times, or equivalent to 15 % Thai RDI. Furthermore, when germinated for a further 72 hours, thiamine content increased up to 4.2 times or equivalent to 50% Thai RDI. It was also found that roasting stages to develop product color and aroma result in rapid loss of thiamine content. However, selecting 170°C for

20 minutes resulted in the least amount of thiamine loss and was most suitable for actual production in benefit/cost terms and provided the best attributes regarding acceptable colour, aroma, and flavor. In this regard, thiamine content increased up to 1.6 times, or the equivalent of 20% Thai RDI, which was considered in the “high/enrich” categories according to Thai RDI criteria.

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