
APST

Asia-Pacific Journal of Science and Technology
<https://www.tci-thaijo.org/index.php/APST/index>

 Published by the Research and Graduate Studies,
 Khon Kaen University, Thailand

Influence of temperature on amylase enzyme profile during germination of two upland rice varieties

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Received 22 February 2021

Revised 13 April 2021

 Accepted 20 May 2021

Abstract

Two upland rice varieties, Khao Niew Dam (KK) and Khao Niew Kiew Ngu (KN) cultivated in Chiang Rai were selected to study. Germination is a key step during which several enzymes are expressed. Three temperatures, 25, 30 and 35 °C, were used during germination to investigate the profiles of α - and β -amylase enzymes. In the case of KK, the maximum specific activity of α -amylase was 2.32 Unit (U)/mg_{protein}, while that of β -amylase was 1.96 U/mg_{protein} at 35 °C (on day 4 and 5). In the case of KN, the maximum specific activity of α -amylase was 1.72 U/mg_{protein} (at 35 °C, day 4), while that of β -amylase was 2.34 U/mg_{protein} (at 25 °C, day 5). The total carbohydrate content of both KK and KN was highest at 30 °C (9.52 and 9.57 g/L, respectively) on day 5 and 4, respectively. The % malting loss was highest in KN at 30 °C on day 5 (22.99 %). The Maximum productivity of reducing sugar ($Q_{p,max}$) of KK at 35 °C was 16.23 g/L.d, while that of KN was 11.87 g/L.d at 25 °C and on day 4. In the case of KN, $Q_{p,max}$ at 25 °C and 35 °C was not different significantly (11.87 and 11.08 g/L.d, respectively). The results of this study have shown that the suitable temperature and duration time for rice germination during malting process of both KK and KN are 35 °C and four days, respectively. Germination temperature and duration time affect the amylase enzyme profile leading to formation of various products.

Keywords: Amylase enzyme profile, Germination, Reducing sugar, Temperature, Upland rice varieties

1. Introduction

Vinegar is a popular product, which contains many bioactive compounds. Vinegar provides several health benefits, such as contributing to weight loss and reduction of belly fat, improving heart health and immunity, controlling blood sugar, as well as, enhancing shiny hair and glowing skin [1]. Vinegar is one of the main products of fermentation of yeast and acetic acid bacteria (AAB). Briefly, it is an acidic liquid containing acetic acid as the main component and made from fermentable carbohydrate sources in two steps; alcoholic fermentation and acetification [2]. In alcoholic fermentation, yeast converts carbohydrates into ethanol, while in acetification, the second-stage fermentation, AAB oxidize ethanol into acetic acid [3]. Generally, vinegar can be produced by various kinds of fruits and vegetables such as grapes and apples. Cereal grains, which contain high levels of carbohydrate, can also be used as raw material for vinegar production. Thus, rice and malt vinegars are produced in some countries [4].

Rice (*Oryza sativa*) is one of the most important cereals in the world, especially in Southeast Asia. It belongs to the Poaceae (Gramineae) or grass family. In Thailand, rice is a staple food and an economically important grain. In 2020, Thailand exported around 5.7 million tons of rice [5]. Rice varieties have different nutritional values and chemical constituents [6,7]. Glutinous rice or sticky rice (*O. sativa* var. *glutinosa*) is a variety of widely consumed rice cultivated in North and Northeast Thailand. Khao Niew Dam (KK) (black glutinous rice) and Khao Niew Kiew Ngu (KN) are two common types of sticky rice found in North Thailand. Both of these varieties have high antioxidant activity, most especially the black rice, which contains anthocyanins [7,8].

Germinated rice or sprouted rice has enhanced nutritional value and significant health benefits. The main nutritional and bioactive constituents found in germinated rice are vitamins, minerals, fiber, amino acids, ferulic acid, γ -oryzanol, and γ -aminobutyric acid (GABA) [9,10]. During the germination process, the rice embryo must be sprouted under suitable conditions. Generally, rice can be germinated by steeping it in warm water (35-40 °C) for about 10-12 h, and keeping it under moist conditions for 20-24 h. Water must be changed every 3-4 h to prevent fermentation and maintain the temperature. This process yields 0.5-1 mm length sprouts from the rice grain. Thus, germinated rice has high nutrient content. Boiling germinated rice is used to extract wort, which serves as the substrate for fermented beverages such as beer, sake and vinegar. The superior nutritional value of germinated rice positively affects the quality of the wort [11,12].

Malting consists of three main processes including steeping, germination and kilning. These processes aim to generate the amylolytic enzymes necessary for hydrolysis of polysaccharides. Two major classes of hydrolytic enzymes are found during malting; α -amylase, an endohydrolase enzyme, which breaks down α -1,4-glycosidic bonds in starch and β -amylase, a saccharifying enzyme, which hydrolyzes starch to produce maltose from the non-reducing end of 1,4- α -glucan [6,13-15]. Kilning is the final step and it stops enzymes activities. However, steeping degree, germination temperature and time greatly affect enzymatic activity [6,16-18]. This study aimed to investigate the effect of temperature during germination on the profile of the two key enzymes, α - and β -amylase. Two upland rice varieties, KK and KN were used as the source of amylolytic enzymes used in the mashing step of wort preparation.

2. Materials and methods

2.1 Raw materials

Two upland rice varieties including KK and KN were bought from a merchant in Chiang Rai, Thailand. Sterile water was used to soak both rice varieties 3 times. After soaking, rice was kept at 4 °C for further experiments.

2.2 Rice grains preparation (pre-steeping)

One kilogram of rice grains was placed in trays. Rice was activated by soaking in 2 L of distilled water and left at 25 °C for 1 h. Rice was then let to dry by air rest at 4 °C for 3 days. Five milliliters of distilled water were sprayed on the rice once a day.

2.3 Effect of temperature during germination step

One kilogram of prepared rice grains was filled into a net bag placed on plastic trays. Rice was germinated in a constant climate chamber (BINDER™ KBF 115, USA) at 90% relative humidity according to Usansa et al. (2011) [19]. Three temperatures 25, 30 and 35 °C, were investigated. The malting program was carried out at each temperature using the steeping and air resting program, which were switched every 6 h until the steeping degree was raised to 44% [19]. During the process, rice grains contained in the net bag were flipped every 1 h and washed using 2 L of fresh distilled water every 3 h. At 44% steeping degrees, rice grains typically started to germinate. Germinated rice, which was contained in a net bag was also flipped and washed as described above. This process was done for 5 days. Fifty grams of germinated rice were collected daily for further analysis. All treatments were performed in duplicate.

2.4 Kilning process (drying step)

Forty grams of germinated rice were dried at 50 °C for 24 h in a hot air oven. The roots of germinated rice were removed before being kept at -20 °C for further analysis. The remaining 10 g of germinated rice were used for moisture content analysis.

2.5 Analysis methods

Qualitative and quantitative parameters of germinated rice grains (malt) were determined. These parameters included α - and β -amylase activity, total protein, moisture content, malting loss, as well as, total carbohydrate and reducing sugar content.

2.5.1 α - and β -amylase assay

The α - and β -amylase assays were performed using by modifying the method of Usansa et al. (2009) [6]. Crude enzyme was extracted from 1 g of ground germinated rice derived from the kilning step mixed with 9 mL

of buffer solution containing 50 mM Tris HCl (pH 7.4), 3 mM CaCl₂ and 4 mM NaOH. The mixture was incubated at 25 °C for 30 min and mixed every 15 min using a vortex mixer. Subsequently, the mixture was centrifuged at 3,000 x g for 10 min. The supernatant or crude enzyme was kept on ice until use (fresh crude enzyme).

To analyze α -amylase activity, 0.5 mL of crude enzyme was incubated at 70 °C for 5 min. Five hundred microliters of 1% (w/v) soluble starch containing 50 mM acetate buffer (pH 5.5) with 0.003% CaCl₂ was added into fresh crude enzyme. The reaction was allowed to proceed for 10 min and terminated by adding 1 mL of 3,5-dinitrosalicylic acid solution (DNS). The mixture was boiled for 5 min followed by addition of 2 mL of distilled water. Absorbance was measured at 540 nm following thorough mixing.

To analyze β -amylase activity, the same procedure as α -amylase assay was followed with some modifications. Briefly, fresh 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 instead.

Given that:

One unit of α -amylase activity is required for the production of 1 μ mole of maltose in 1 min at 70 °C and pH 5.5;

One unit of β -amylase activity is required for the production of 1 μ mole of maltose in 1 min at 55 °C and pH 3.6;

Specific enzyme activity was calculated according to Equation no. 1 below:

$$\text{Specific enzyme activity (U/mg}_{\text{protein}}) = \frac{\text{Enzyme activity (U/mL)}}{\text{Protein content (mg/mL)}} \quad (1)$$

2.5.2 Reducing sugar analysis

The amount of reducing sugar present in germinated rice grains was analyzed according to Miller (1951) [20]. One gram of rice powder derived from the kilning process was dissolved in 10 mL distilled water and mixed thoroughly. The mixture was incubated at 20 °C for 30 min. The supernatant was separated by centrifugation at 3,000 x g for 10 min. Five hundred microliters of supernatant was mixed with 0.5 mL of DNS solution. The reaction was executed at 95 °C for 5 min and left to cool for 5 min. Absorbance was measured at 540 nm using a spectrophotometer (Thermo Scientific GENESYS 20, USA). The calibration curve was prepared using standard maltose (0.2-1.0 μ mol/mL) and the reducing sugar concentration was calculated according to the linear equation obtained from this calibration curve.

2.5.3 Total carbohydrate analysis

The concentration of total carbohydrates was analyzed using the phenol-sulphuric acid method [21]. One gram of rice powder derived from the kilning process was dissolved in 10 mL distilled water and mixed thoroughly. The mixture was incubated at 20 °C for 30 min. The supernatant was separated by centrifugation at 3,000 x g for 10 min and 2.7 mL was hydrolyzed in 0.018 mL of concentrated hydrochloric acid for 15 min. The mixture was neutralized with sodium carbonate until pH reached 7.0. The volume was adjusted to 6 mL and centrifuged at 3,000 x g for 10 min. The mixture consisting of 0.2 mL supernatant, 0.2 mL 5% phenol and 1 mL concentrated sulfuric acid was added into a test tube and incubated for 20 min at 30 °C in a water bath. Absorbance at 490 nm was measured using a spectrophotometer (Thermo Scientific GENESYS 20, USA). The calibration curve was prepared using standard glucose (0.2-1 g/L) and the concentration of total carbohydrate was calculated according to the linear equation obtained from this calibration curve.

2.5.4 Total protein analysis

Total protein content present in germinated rice grains was analyzed by modifying the method of Usansa et al. (2009) [6]. Crude protein was extracted using 1 g of ground malt derived from the kilning process mixed with 9 mL of buffer solution containing 50 mM Tris HCl (pH 7.4), 3 mM CaCl₂ and 4 mM NaOH. The mixture was incubated at 25 °C for 30 min and mixed every 15 min using a vortex. Subsequently, the crude extract was centrifuged at 3,000 x g for 10 min. The supernatant was kept on ice until further use (fresh extract).

The total protein content was quantified using Lowry's assay [22]. Briefly, 0.2 mL of crude protein was added into different test tubes along with 2 mL of alkaline copper sulphate reagent. The mixture was incubated at room temperature for 10 min. Subsequently, 0.2 mL diluted Folin Ciocalteu solution was added into each tube and incubated for 30 min. The absorbance was measured at 660 nm by using a spectrophotometer (Thermo Scientific GENESYS 20, USA). The calibration curve was prepared using standard Bovin Serum Albumin (BSA) (2-10 mg/mL) and the concentration of total protein content was calculated according to the linear equation obtained from this calibration curve.

2.5.5 Moisture content analysis

The moisture content was determined according to Usansa et al. (2009) [6]. Germinated rice grains were placed on a paper towel for 5 min to drain excess water. Five grams of drained germinated rice grains were placed into moisture cans and dried in a hot air oven at 105 °C for 3 h. The moisture content was calculated according to Equation no. 2 below.

$$\text{Moisture content} = \frac{(W_1 - W_2)}{W_1} \times 100, \quad (2)$$

where W_1 and W_2 represent the weight of drained germinated rice grains (g) and dried germinated rice (g), respectively.

2.5.6 Malting loss analysis

The malting loss of germinated rice was determined by modifying the method of Usansa et al. (2009) [6]. The percentage of weight loss was calculated according to Equation no. 3 below.

$$\text{Malting loss} = \frac{(R - M)}{R} \times 100, \quad (3)$$

where R and M represent the weight of 100 rice grains and 100 malted rice, respectively.

2.5.7 Statistical analysis

Statistical analysis was performed using Microsoft Office 2010 (Microsoft Corporation, USA). Results were expressed as means $\pm$ SD using SPSS software (version 20.0). All data on amylase enzyme activity, protein content, total carbohydrate content, reducing sugar and productivity of reducing sugar formation were normalized. All differences among treatments were statistically evaluated using one-way ANOVA and compared by Duncan's Multiple Range Test (DMRT). Values of $p \leq 0.05$ were considered as statistically significant.

3. Results and discussion

3.1 Effects of temperature on amylase enzyme profile and protein content

Amylolytic enzymes play an important role in the malting process. The α - and β -amylase along with α -glucosidase work synergistically to digest stored carbohydrates into fermentable sugars, a step that is necessary for wort production [23]. This experiment was focused on the two major enzymes, α - and β -amylase, and their specific activity. Two upland rice varieties, KK and KN, were used as the raw materials and the activity of the two above mentioned enzymes was measured. The specific enzyme activities of α - and β -amylase in KK were 0.52 and 0.34 U/mg_{protein}, respectively, while in KN, the measurements were 0.49 and 0.64 U/mg_{protein}, respectively. During the germination step, activity of α -amylase and β -amylase increased in both upland rice varieties (Figure 1). For the germination step of KK, the maximum specific enzyme activity of α -amylase was 2.32 U/mg_{protein}, while that of β -amylase was 1.96 U/mg_{protein} noted at 35 °C on day 4 and 5 of germination, respectively. Meanwhile, the maximum specific enzyme activity of α -amylase and β -amylase of KN were 1.72 and 2.34 U/mg_{protein} noted on day 4 and 5 of germination under 35 °C and 25 °C, respectively (Figure 1). At the end of the process, the specific enzyme activity among the three temperatures varied significantly. In both upland rice varieties, the specific enzyme activity of α -amylase was significantly higher at 35 °C than at 25 and 30 °C, whereas that of β -amylase was significantly higher at 35 °C in the case of KK and at 25 °C in the case of KN (Table 1). High temperature causes starch degradation, thus the thermal treatment used during incubation in this study may have enhanced the enzymatic hydrolysis of starches [18]. However, Usansa et al. (2009) [6] reported that the optimal temperature for maximum activity of amylolytic enzymes from six Thai rice cultivars was 30 °C.

Generally, the amylolytic enzyme mechanism in rice grain comprises hydrolysis of two distinct polysaccharides: amylose (linear, made of α -1,4-linked glucans) and amylopectin (linear, made of α -1,4-linked glucans branched with α -1,6 linkages) [24]. The three main amylolytic enzymes are α -amylase, β -amylase and glucoamylase (α -glucosidase). The α -amylase enzyme is an endoamylase, which digests only the α -1,4-bonds in the inner regions of the starch molecule. This mechanism causes a rapid loss of the starch solution [25]. In the absence of α -amylase, starch is not degraded [26]. The β -amylase enzyme is an exoamylase, which also digests the α -1,4-bonds, but it acts on the non-reducing end of the molecule as well. Glucoamylase breaks the α -1,6-bonds and produces only small molecular products, e.g. glucose [27]. According to the results of this study (Figure 1), at 25 °C the specific enzyme activity of β -amylase was higher than that of α -amylase. In contrast, the specific

enzyme activity of α -amylase was higher than that of β -amylase at both 30 and 35 °C. Guglielminetti et al. (1995) [28] and Magneschi & Perata (2009) [26] reported that α -amylase plays a key role in starch hydrolysis of rice grain under anoxia (absence of oxygen), a condition that might have been reached at the higher temperatures used herein, possibly due to the faster rate of oxygen depletion and longer steeping duration [6]. However, to clarify the results, α -glucosidase must also be introduced to complete the amylolytic enzyme reaction.

Protein content was positively correlated with amylase enzyme levels. Both time and temperature during germination affected protein content (Figure 2). Specifically, the initial protein content of KK and KN varieties was 12.84 and 9.34 mg/mL, respectively. At the end of the germination step, the highest protein content in both KK and KN (17.55 and 14.38 mg/mL) was noted at 30 °C, whereas the minimum protein content was found at 35 °C in both varieties (Figure 2). However, it is not only enzymes that are represented in terms of total protein, but also other storage proteins. Usansa et al. (2009) [6] demonstrated a correlation between protein content and water uptake in rice. Specifically, the authors found that protein content correlated to steep-out moisture content, whereby higher protein content provided more water absorption, which in turn could stimulate enzymatic activity in the rice grain. Temperature of germination dictates activity level of the key enzymes, which will be re-activated in order to produce fermentable sugar in the mashing step of wort preparation.

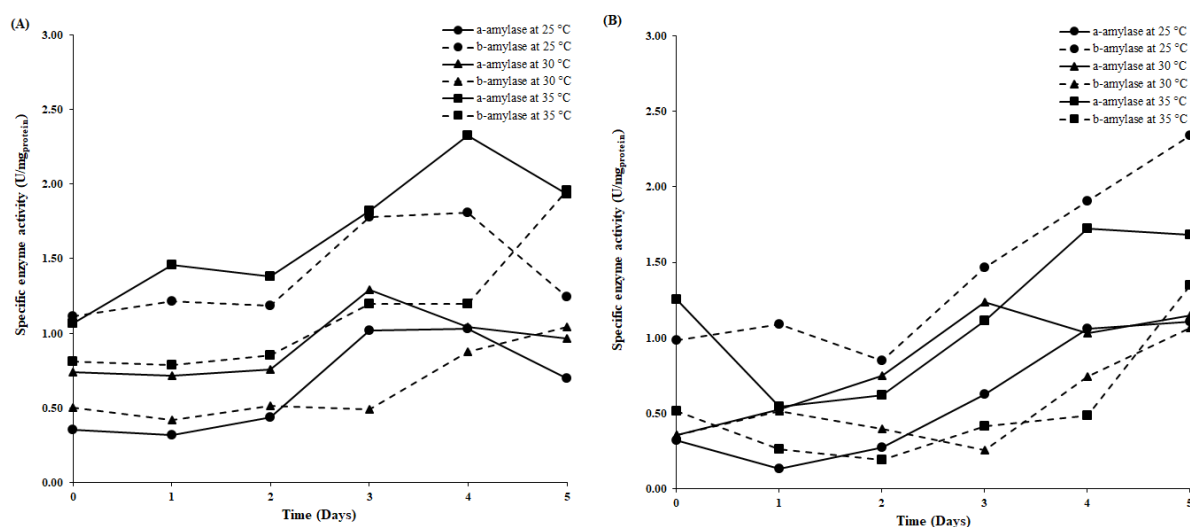


Figure 1 The influence of temperature on amylase enzyme profile (specific enzyme activity, U/mg_{protein}) during germination of two upland rice varieties (A) Khao Niew Dam (KK) and (B) Khao Niew Kiew Ngu (KN). The α - and β -amylase enzymes are shown as a solid and dash line, respectively.

Table 1 Comparison of specific enzyme activity of α - and β -amylase enzyme profiles and protein content on day 5 of germination of the two upland rice varieties Khao Niew Dam (KK) and Khao Niew Kiew Ngu (KN) at different temperatures.

Parameters	KK			KN		
	25 °C	30 °C	35 °C	25 °C	30 °C	35 °C
Specific enzyme activity						
α -amylase	0.70±0.27 ^b	0.96±0.39 ^b	1.94±0.33 ^a	1.11±0.20 ^b	1.15±0.14 ^b	1.68±0.51 ^a
β -amylase	1.25±0.29 ^b	1.04±0.32 ^b	1.96±0.20 ^a	2.34±0.34 ^a	1.07±0.22 ^b	1.35±0.73 ^b
Protein content	17.39±5.03 ^a	17.55±4.24 ^a	7.46±0.70 ^b	7.71±0.57 ^b	14.37±1.45 ^a	4.86±0.08 ^c

Values were given as mean value ± standard deviation of four replicates.

The different superscript italic letters indicate significant difference values in the same row at $p \leq 0.05$.

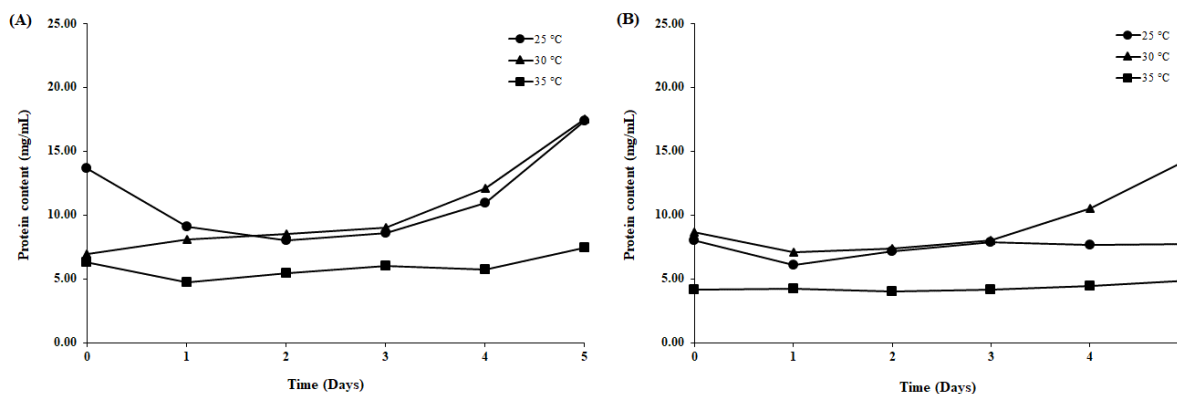


Figure 2 The influence of temperature on protein content during germination of two upland rice varieties: (A) KK and (B) KN.

3.2 Effect of temperature on moisture content, malting loss, total carbohydrate content and reducing sugar

The moisture content, malting loss, total carbohydrate content and reducing sugar during the germination step of malting process at three temperatures (25, 30 and 35 °C) of two upland rice varieties, KK and KN, were studied. During germination, the moisture content was measured without controlling. The moisture content was highest in both upland rice varieties (53.63 and 51.01% found in KK and KN, respectively) at 30 °C on day 5. At 35 °C moisture content in KK and KN was 47.50 and 42.86%, respectively, while at 25 °C, the content was 46.20 and 45.53% found in KK and KN, respectively. This study has shown that water absorption of rice grains can be affected by temperature. Previous studies have shown that increasing the temperature to suitable levels increases water absorption during rice germination and also enhances many metabolic activities in the rice grain [6]. However, high temperature causes inhibition of water absorption by preventing cell wall loosening of the embryo, which is found in some cereals including rice grain [29-31]. In this experiment, the moisture content present in KK was higher than that of KN. Usansa et al. (2009) [6] reported that the difference of moisture content depends on the variety of rice, its properties and the steeping temperature (especially at 30 °C). In addition, the effect of appropriate temperature on water absorption could enhance metabolism of germination of rice [32].

The % malting loss was defined as the use of matter in the embryo, which uses the products of enzyme hydrolysis as substrates [33]. The results of this study indicated that the malting loss of germinated rice was affected by duration and temperature during germination. Malting loss was slightly increased day by day until the end of the process. At 35 °C, KK showed the highest % malting loss than at 25 and 30 °C. Meanwhile, the % malting loss was highest at 30 °C than at 25 and 35 °C for KN (Figure 3). These results are in agreement to those noted during malting of six Thai rice cultivars [6]. Besides % malting loss, total carbohydrate content can also be used to represent the substrate consumption during amylase enzyme hydrolysis. The phenol-sulphuric acid method was used in order to monitor change of total carbohydrates including glucose, galactose and mannose. In this method, carbohydrates undergo a dehydration reaction to form furan derivatives then condense with phenolic compounds to produce dark colored complexes. Absorption at 490 nm indicates presence of hexoses such as glucose, galactose and mannose [34]. The results indicated that the total carbohydrate content during germination varied with time and temperature. The initial total carbohydrate content present in KK (1.35 g/L) and KN (1.81 g/L) was analyzed. At 30 °C, KK and KN had the higher total carbohydrate content than at 25 and 35 °C. In terms of time, the highest total carbohydrate content was 9.52 and 9.57 g/L on day 5 and 4 of germination, respectively (Figure 4). The total carbohydrate content at day 5 of germination of both upland rice varieties is shown in Table 2. However, the increase of total carbohydrate was likely due to the formation of other sugars caused by enzyme hydrolysis as mentioned above. Carbohydrates are mainly digested during seedling development [35]. The most abundant carbohydrate in rice is starch, a compound that decreases rapidly during germination by enzymes and positively correlates with reducing sugars. Degradation is the result of amylase activities to produce sugar, such as glucose and maltose [18]. An increase of total carbohydrate concentration was observed during germination of rice grains, which is likely due to starch hydrolysing into simple carbohydrates e.g. glucose [36].

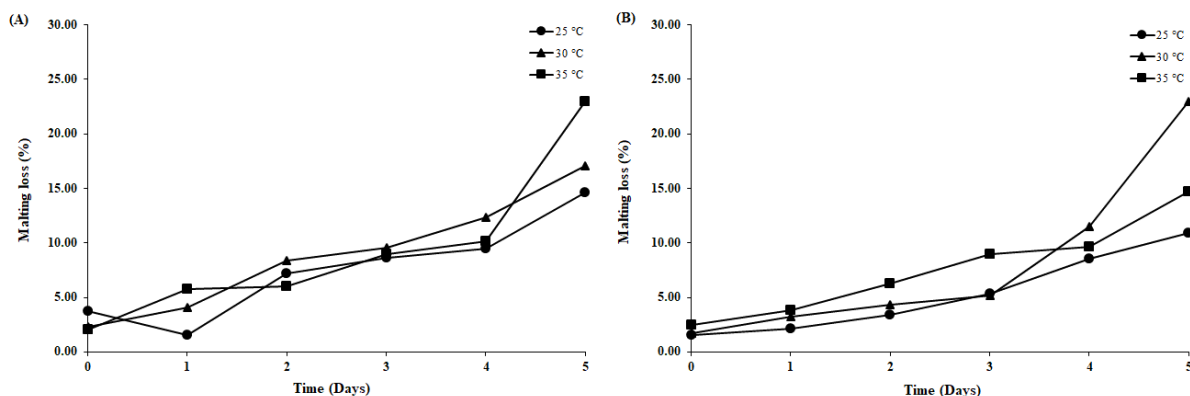


Figure 3 The influence of temperature on % malting loss during germination of two upland rice varieties (A) Khao Niew Dam (KK) and (B) Khao Niew Kiew Ngu (KN).

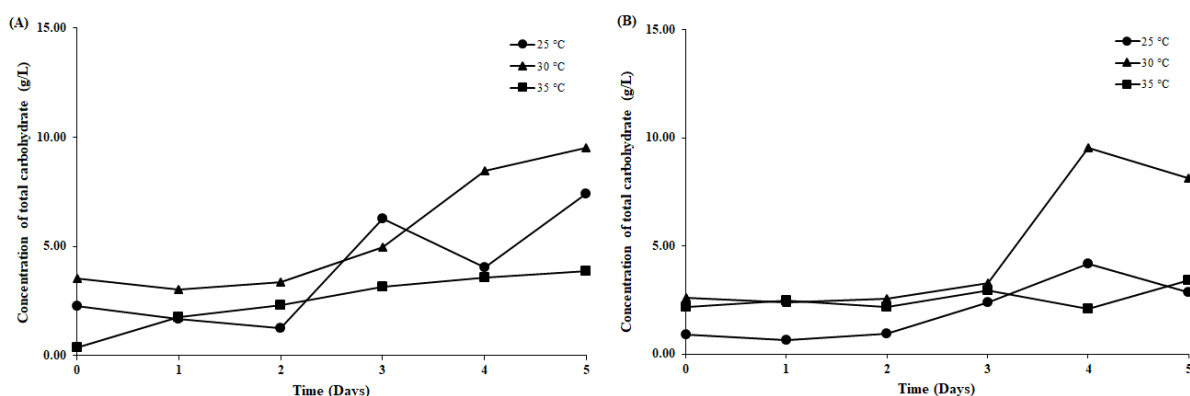


Figure 4 The influence of temperature on total carbohydrate content during germination of two upland rice varieties (A) Khao Niew Dam (KK) and (B) Khao Niew Kiew Ngu (KN).

The changes of sugar content at different temperatures were also investigated during the germination step of malting process. Sugar content was defined as the product of hydrolysis by amylolytic enzymes, which increased during germination until it was approximately equal to total carbohydrate content [37]. The initial reducing sugar concentrations of KK and KN were 16.18 and 18.52 g/L, respectively. In this study, reducing sugar increased in all treatments and both upland rice varieties. Interestingly, KK had higher reducing sugar concentration than KN in all treatments (Figure 5). The highest reducing sugar concentration was found on day 5 of germination at 25 °C in both KK and KN at 102.94 and 78.29 g/L, respectively (Figure 5, Table 2). Productivity (Q_p) is used to indicate the efficiency of the process [38]. Herein, the maximum productivity of reducing sugar ($Q_{p,max}$) was found at 35 °C in KK (16.23 g/L.d) on day 4 of germination and at 25 °C in KN (11.87 g/L.d) on day 4 of germination (Table 2). The Q_p of KN at day 4 of germination between 25 °C ($Q_p = 11.87$ g/L.d) and 35 °C ($Q_p = 11.08$ g/L.d) was not significantly different ($p \leq 0.05$) (Table 2). Based on these results, high α -amylase activity can be obtained if the germination process lasts for 4 days in the KN variety (Figure 1(B)). The $Q_{p,max}$ of reducing sugar of KK was noted at 35 °C (16.23 g/L.d) on day 4 of the germination and was the highest among treatments, suggesting that this condition is suitable for germination. Even though the maximum product concentration was not reached, this condition was suitable for product formation. According to the results, the temperature and rice variety had a significant effect on the total carbohydrate content, reducing sugar concentration and Q_p (Table 2). Since α - and β -amylase enzymes are critical for starch hydrolysis, Q_p , which represents the activity of these enzymes, is an important criterion for selecting the suitable temperature. Comparing between KK and KN varieties, the former germinated at 35 °C for 4 days and showed the highest Q_p , which corresponded to α - and β -amylase activity as described above. These results suggest that a 35 °C temperature and duration time of four days are suitable conditions to induce metabolism of rice grains of both KK and KN varieties during the germination process [39].

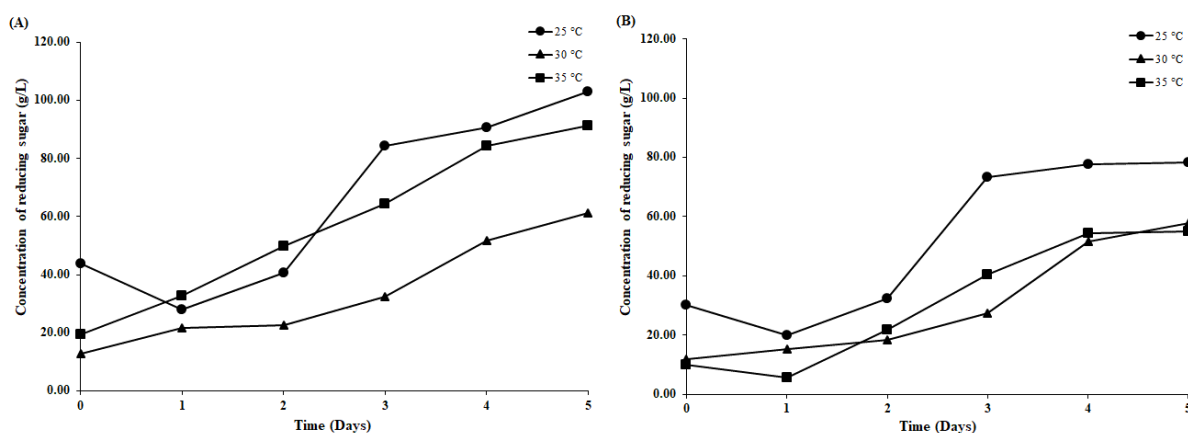


Figure 5 The influence of temperature on reducing sugar concentration during germination of two upland rice varieties; (A) Khao Niew Dam (KK) and (B) Khao Niew Kiew Ngu (KN).

Table 2 Comparison of total carbohydrate and reducing sugar concentration and productivity of reducing sugar on day 5 of germination of two upland rice varieties (Khao Niew Dam (KK) and Khao Niew Kiew Ngu (KN)) at different temperatures.

Parameters	KK			KN		
	25 °C	30 °C	35 °C	25 °C	30 °C	35 °C
Concentration						
Total carbohydrate	7.43±0.55 ^b	9.52±1.39 ^a	3.88±1.88 ^c	2.89±0.09 ^c	8.13±1.06 ^{ab}	3.43±0.85 ^c
Reducing sugar	102.94±5.99 ^a	61.09±8.13 ^c	91.38±5.63 ^a	78.29±16.93 ^b	57.81±0.49 ^c	54.90±4.27 ^c
Productivity of reducing sugar (Q_p)						
$Q_{p,5}$	11.82±1.77 ^b	9.68±1.30 ^{bc}	14.40±1.27 ^a	9.61±2.99 ^{bc}	9.22±0.26 ^{bc}	8.99±1.12 ^c
$Q_{p,max}$	13.49±3.20 ^{ab}	9.75±2.10 ^c	16.23±3.90 ^a	11.87±3.41 ^{bc}	9.99±3.55 ^c	11.08±1.96 ^{bc}
	(day 3)	(day 4)	(day 4)	(day 4)	(day 4)	(day 4)

Values are given as mean value ± standard deviation of four replicates.

The different superscript italic letters indicate significant differences in values in the same row at $p \leq 0.05$.

$Q_{p,5}$ and $Q_{p,max}$ were calculated based on whole germination time (until day 5 of germination) and the maximum reducing sugar concentration, respectively.

4. Conclusion

Temperature has an effect on amylase enzyme profile during the germination step of malting process. The activity of α - and β -amylase fluctuated along with the germination time. However, this study suggested that a temperature of 35 °C and duration time of 4 days of germination were the most suitable conditions to get high α - and β -amylase activity and $Q_{p,max}$ in both KK and KN varieties. This was due to the activity of the key enzyme of starch degradation, α -amylase, which had the highest activity under these conditions. Temperature and duration of germination affected malting loss and total carbohydrate content according to the reaction of key enzymes in starch hydrolysis. In order to get the suitable levels of those key enzymes to utilize in mashing step of wort preparation, the appropriate temperature and duration of rice germination must be investigated. Thus, temperature used during is the key factor, which should be customized according to each rice variety.

5. Acknowledgements

Financial support for this study was provided from the National Research Council of Thailand and Mae Fah Luang University, both of which are gratefully acknowledged. Instrument use was supported by School of Science and Scientific and Technological Instruments Center, Mae Fah Luang University. The authors wish to thank Dr. Sunita Chamyuang, Dr. Natsaran Saichana and Dr. Kitiphong Khongphinitbunjong for useful discussions on this work.

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