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Influence of the Kilning Conditions on Enzymatic Activity of Rice (*Oryza sativa*) Malt

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Abstract : This study investigated the effect of kilning condfition on the diastatic power and activities of protease, α-amylase, and β-amylase in rice malt. Common rice (*Oryza sativa*) was steeped at 30°C for 50 h, germinated at 30°C for 7 days, and kilned at 50°C for 24 h. The moisture content and enzymatic activities were determined under various kilning times. As a result, the moisture content was reduced from 42.1% to 3.9% after 24 h of kilning at 50°C. The protease activity of rice malt showed lower value than that of barley malt. All enzymatic activities were decreased during the kilning stage. Results indicated that after prolonged kilning at 50°C, the inactivation of hydrolytic enzymes might be occurred. Even though the amylolytic activity of malted rice showed low value, the rice malt shows the potential characteristics as ingredient for the brewing and cereal industries.

Keywords : Rice, enzyme activity, kilning, malting, protease activity, diastatic power, β-amylase, α-amylase activity.

1. Introduction

Drying process is used with the aims to keep the enzymatic activity of agricultural products [1-4]. It involves removal of volatile substances (commonly, but not exclusively, water) from a solid product, and it is a process in which the water activity of a product is decreased due to removal of water by vaporization [3-6]. Thus, the drying process is the conjunct of science and technology that needs of experiments on several phenomena that occur in this process [7]. Some considerations about the initial and moisture contents of product are used to justify the drying phenomenon. These considerations are the form of water transport into the solid structure and surfaces [8]. Rice (*Oryza sativa*) is an agricultural product, very popular in Vietnam, and low

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cost price. Thus, the rice malt obtaining will go aggregate valor to rice culture. The enzyme production and the malting process may differ between rice and barley.

The aim of the present work is to analyse the effect of kilning on the moisture contents, enzymatic activity, specifically α-amylase, β-amylase, diastatic power, and protease activity during the malting of rice. The enzyme levels of malted rice were compared with those of malted barley. As a result, the α-amylase and β-amylase activities in malted rice showed lower values than those of malted barley. The maximum activity level of α-amylase was 47.8 units.

2. Experimental

2.1 Materials

The rice (*Oryza sativa*, OM4088) was supplied by the Cuu Long Delta Rice Research Institute (Cantho Province, Vietnam). OM4088 was harvested in 2006. For the malting process, the commercial rice *(Oryza sativa)* samples were malted by the steeping process for 50 h. After the steeping process, the malt with about 40 wt% of moisture was wrapped on the cheese cloth, kept in the perforated plastic trays, and germinated in the dark at 90±5% RH. The malt was dried at 50°C for 24h. The convective dryer with air circulation was used for the rice malt drying process. The sprout and rootlet of samples were removed for analysis process.

2.2 Analytical procedures

Moisture content: The samples were analyzed for triplicate and the results were validated statistically by the mean ± standard deviation. The determination of moisture was described by the Nzelibe and Nwasike method [9].

Diastatic power α-amylase, β-amylase, and protease activity: Amylase activity was analyzed by the Bernfeld methods [10, 11]. The flour sample was extracted with acetate buffer (pH 4.3) for 1 h at ambient temperature (about 20°C). The amylase activity of extract was expressed as maltose units and defined as the amount of maltose (mg). The amylase activity of extract released by the action of malt enzyme extracted from 1 g of malt flour in acetate buffer (pH 4.3) on soluble starch at 40°C for 30 min. For α-amylase and β-amylase, the joint activity of α- and β-amylases were determined by a modification of the American Society of Brewing Chemists methods [12]. The activity of α-amylase was determined by destroying the β-enzyme by heat inactivation making allowance for the concomitant but minor amount of inactivation of the α-enzyme. The β-amylase activity was calculated as a difference between the original activity and the corrected α-amylase activity. Those results were expressed as an activity of the created maltose in the malt for one hour (mg/g.h) [13]. The protease activity level was measured according to the Wilstester method [12].

Mashing: The slurry was heated in the water bath and deposition for 30 min. The supernatant liquid was decanted and the starch was heated until achieved gelatinization state. The solution was cooled and mixed with the supernatant liquid. One third of the mash was again heated, mixed with the main mash, and the mash matrix was reheated to 65-70°C for 60 min. The pH of the mash was adjusted to 5.6 with 10% of citric acid solution. One half of the mash was boiled and mixed with the main of mash. The temperature of the mash was increased from 65-70°C for 30 min and 70-75°C for 30 min [3].

Wort: The free alpha amino nitrogen (FAN) samples were performed by the Taylor & Boyd method [12]. The FAN contents were measured by the Ninhydrin method [12] (the Ninhydrin method was as

outlined in the Institute of Brewing Method of Analysis (IOB 1989) and with some modifications by Ezeogu in 1996). The protein in malting and the soluble nitrogen in extract were measured by the semi-micro Kjeldahl distillation method [11, 12]. The extract (%) and colour of worts were measured in accordance with the EBC method. The mash was filtered by the filter paper with the graduated cylinders (Schleicher & Schnell, Germany). The time taken for each wort samples through the filter was recorded.

3. Results and Discussion

3.1 Moisture contents

The effect of time on moisture contents during rice kilning is presented in Fig. 1. The temperature of kilning process was 50°C for 24 h. During the free drying stage of kilning [5], the moisture content of rice malt was decreased from 42.1% to 25.5% after 2 h of kilning. As the intermediate stage of kilning begins, the rate of drying begins to slow down due to the physically or the chemically bound nature of residual moisture, which restricts evaporation (Fig. 1). For the rice, similar results were obtained to those of barley malt kilning [5] where the moisture contents was decreased from 42.1% to 10.2% after 6 h of kilning. The final stage of barley

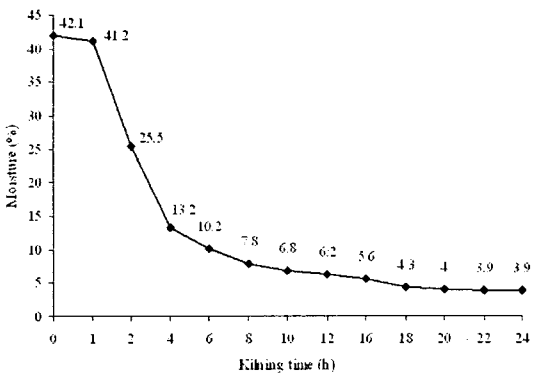

Fig. 1. The moisture percentages (%) vs. kilning times (h).

malt kilning was calculated by the removal of firmly bound water in the grain. The water content was reduced from 10.2 % to 4%. The results showed that in the rice malt, the rate of drying was slowed, and the moisture contents was decreased from 10.5% to 4.0% (final moisture contents) after 24 h of kilning at 50°C.

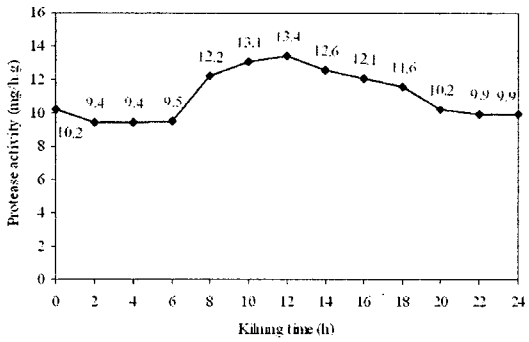

Fig. 2. The protease activity of rice malting (mg/h.g) vs. kilning time (h).

3.2 Protease activity

A variety of endo and exo-proteases have been identified in barley green malt [14]. In this study, the protease activity level was measured with gelatin as a substrate, which gives an indication of the total proteolytic activity level of grains. The changes in protease activity during the kilning of rice malting are showed in Fig. 2. The proteolytic activity was decreased by 10.2 to 9.4 mg/h.g after the first of kilning process (4 h). This correlates with the result of Dickson and Shands *et al.* [15], where a reduction of proteolytic activity in the barley malt was observed in the first few hours of drying at 45°C. This reduction may be due to protease (endo-peptidase) enzymes being inactivated during kilning, since malt endo-peptidases are relatively heat labile and easily inactivated during the kilning process [14]. The proteolytic activity was increased by 9.4 to 13.4 mg/h.g after the second of rice kilning process (4-12 h). This increase can be explained by the beginning of proteolysis

initiated by a slight temperature rise within the grain bed. Taylor and Boyd *et al.* have reported that the proteolysis occurs optimally at 43°C to 50°C for sorghum malts [17]. Alternatively, the increase may be due to the presence of exo-peptidases. Lewis and Young *et al.* have proclaimed that the exo-peptidases with high stability of heat and remained in the endosperm after the kilning process [16]. Our result related with the report of Dickson and Shands *et al.* [15]. The final of barley malt kilning process had week and no effect on the proteolytic activity. Using rice for the malt process, the proteolytic activity was increased (Table 1) and had similar values with the result of Dennis *et al.* [2]. The proteolytic activity was 9.9 mg/h.g after the kilning process. Most of the protease was synthesized during germination [5] and the remained protease was activated during kilning. The proteolytic activity of rice green and barley malts were 10.2 and 7.1 mg/h.g, respectively. The proteolytic activity of barley malt was lower than that of the rice malt (Table 1).

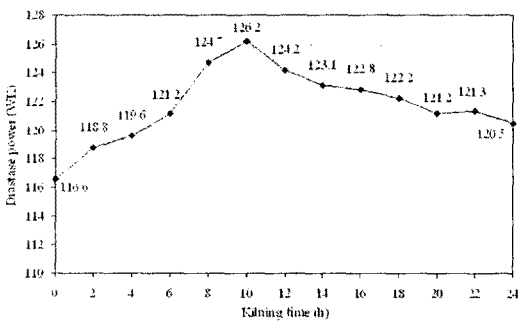

Fig. 3. The enzyme activity of amylase vs. kilning time.

3.3 Diastatic power

Sabramanian *et al.* have analyzed that the most important characteristics of a good malt are high enzyme levels to degrade starch and to obtain a high extract yield [18]. The α-amylase activity of barley malt is a suitable process and the hydrolysis was a completed process after the end of mashing process. Okolo *et al.* have suggested that the temperature of gelatinization may effects on the amylase [13]. It was concluded that the rice malts was used in this study had very low diastatic power when compared with that of the barley malt. The diastatic power was increased with increasing drying time from 0.0 to 10 h and was decreased with long drying time (above 10 h). The diastatic power after 10 h of kilning process (126.2 WK) demonstrated the highest value than those of other samples. The lowest diastatic power was 116.6 WK with the beginning of kilning process. The diastatic power of malted rice is shown in Table 1. The final activity value of malted barley was 75% greater than that of malted rice. The final activity value of malted barley and malted rice were 210 and 123 WK, respectively.

β-Amylase activity

β-Amylase is a heat labile enzyme [16] present in unmalted barley in a bound form (linked *via* disulphide bridges), and a free form and latent form [14]. During malting proteolytic enzymes cleave the disulphide bridges, solubilising the bound β-amylase [19]. In order to determine the amount of total and soluble β-amylase activity, cysteine was used to free the bound enzyme. The β-amylase activity vs. kilning time is presented in Fig. 4. The determined β-amylase activity of unmalted and malted rice are showed in Table 1. The temperature and duration of kilning effected on the amylase activity in sorghum malts [8].

In the fist of kilning process, the β-amylase activity was increased by 650 to 743 mg/h.g with increasing the time of kilning from 0.0 to 6 h. This result related with the report of Okungbowa *et al.* [6]. For the sorghum, the enzyme denaturing phase was avoided and increased enzyme development with the kilning process at low

temperature (i.e. 40°C) [20]. In addition, the possible proteolytic activation of β-amylase zymogens during the enzymaticphase of malt kilning may in part account for the increase in β-amylase activity [16, 19-22]. With the long time of kilning process (above 8 h), the β-amylase activity decreased with increasing the kilning times (from 735 mg/h.g for 8 h to 711 mg/h.g for 24 h). One report has proclaimed that the fraction of β-amylase activity was inactivated event with the suitable thermal-ability and low temperature of conditions [20].

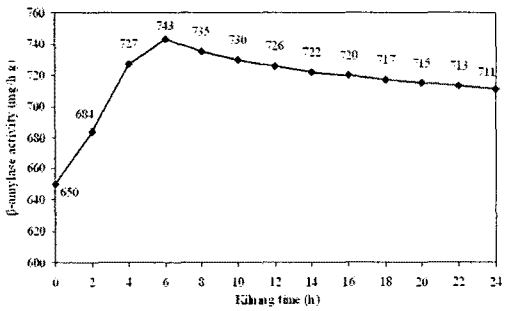

Fig. 4. β-Amylase activity (mg /h.g) of rice malting vs. kilning time (h).

The interdependence of the β-amylase activity on the unmalted and malted rice are presented in Table 1. As our result, the rice produced β-amylase activity during the steeping and germination, and as the same result of Wijngaard *et al.* [23]. Unmalted and malted rices, and malted barley containing β -amylase activity after the kilning process were 134, 711 and 2100 mg/h.g, respectively. The β-amylase activity of rice malt showed lower values than that of barley malt. The β -amylase activity of green malt was 90% greater than that of the rice. However, the β -amylase activity of barley kilning was decreased by 40 % as compared with that of the rice at the final of kilning process [4]. Correspondingly, the optimal for the kilning process of malted rice was found to be 50°C for 24 h.

α-Amylase activity

The relationship between the α-amylase activity of rice and kilning time are demonstrated in Fig. 5. After the first of kilning process (0-18 h), the α-amylase activity was increased from 1235 to 1378 mg/h.g, and as the same value of the Uriyo and Eigel *et al.* reports [24]. The author have indicated that the sorghum αa - amylase was stable during 5-10 h of the drying times at low temperatures. The increase in enzymatic activity could be attributed to continued germination during drying at low temperatures [2]. As our result, with the long time kilning process (18-24 h), the α-amylase activity was decreased (1378 to 1351 mg/h.g).

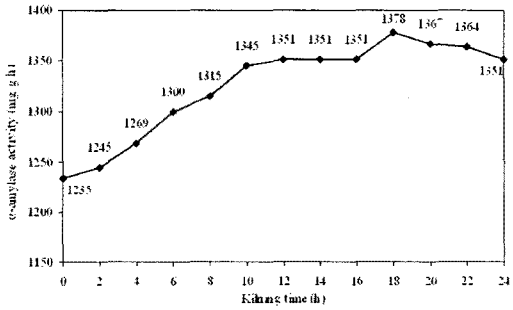

Fig. 5. α-Amylase activity (mg/h.g) of rice malting in kilning time (h).

The heat denaturation effected on the enzymatic activity of sorghum after 10 h of the kilning process. Thus, the enzymatic activity of sorghum was decreased [13]. For this reason, it is referred to as the enzyme inactivating phase of the kilning process [7]. As the same result of barley amylase, the thermal-stability of α-amylase was greater than that of the β-amylase [4]. The heat denaturation effected on the both of amylases. Thus, the amylase showed an increase in inactivation after 7-8 h of kilning process. As the same of unmalted barley results, the unmalted rice contained very low α-amylase activity.

The production of α-amylase was produced during germination. The α-amylase activity of malted rice is shown in Table 1. The final activity of malted barley and malted rice were 1120 and 1351 mg/h.g, respectively. The final activity of malted barley showed lower values than that of malted rice. There were two relationships presented that the relationship between the enzymatic activity of α-amylase and the kilning process, and the relationship between the enzymes and themolecular weight of protein [4]. As a result of heating during kilning, the structure of protein was changed to some denatured materials. The relative enzymatic activity of α-amylase at the end of germination and at the end of kilning was analyzed. As those results, at the end of killing, the α-amylase activity of green malt and barley malt were improved by 9.3 and 15% (after the end of germination), respectively [4].

3.4 Congress mashing

Congress mashing is an essential part of routine malt analysis and as showed in Table 1. The colour of rice and barley worts were 7.1 and 7.5 EBC (European Brewing Convention), respectively. The rice and barley worts extracted by hot water were 67.3 and 75%, respectively.

3.5. Nitrogenous compounds

Nitrogenous compounds affects on the foam, mouthfeel, and tendency to form haze in the final beer. The protein of barley was higher than that of the rice. Accordingly, the free amino nitrogen (FAN) obtained from rice malt showed lower values than that of barley malt. Commercial beer with high FAN contents has an effect on the quality of produce.

4. Conclusions

In this work, the effects of kilning process on the moisture content, enzymatic activity, diastatic power, α-amylase, β-amylase, and protease activity during the malting of rice were investigated. The inactivation of α -amylase activity was greater than that of β -amylase and protease with longer kilning time at 50°C. However, the kilning process has strongly effect on the quality of produce.

Table 1. Physical and enzymatic properties of unmalted rice, malted rice (OM4088), and malted barley

Properties	Unmalted rice	Malted rice	Malted barley
Moisture content (%)	11.5	3.8	6.4
Hot water extract (%)	-	67.3	75
Colour (EBC units)	-	7.1	7.5
Malting loss (%)	-	22.7	-
Free amino nitrogen (%mg/g)	-	266	350
Soluble nitrogen (%)	-	0.41	0.5
Protein (%)	8.65	7.4	8.8
KI index (%)	-	34.5	36.8
Diastatic power (WK)	26	123	210
α-amylase (mg/g)	75	1351	1120
β-amylase (mg/g)	125	741	2100
Proteas (mg gelatin/h.g)	4.2	9.9	7.1

Accordingly, the study of the effects of two-stage or three-stage kilning regime on the properties of produce are important results, and for short time periods. The optimal kilning process for malt and wort produced from the rice was found to be 50°C for 24 h, which ensured survival of the enzymes.

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