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Enzymatic Activity of Liquid *Nuruk* according to Types of *Nuruk* Molds and Degree of Rice-polishing

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ABSTRACT

This study was activity of α-amylase, glucoamylase of liquid *Nuruk* prepared using liquid *Nuruk* (NK) and *Aspergillus kawachii* (AK), *Aspergillus niger* (AN), *Aspergillus oryzae* (AO), *Monascus kaoliang* (MK). To investigate the relationship between the enzymatic activity and the total sugar content of liquid *Nuruk* depending on the types of *Nuruk* molds and the degree of rice-polishing. The activity of α-amylase depending on the types of *Nuruk* molds was shown to be 8.82, 8.72 units/mL in AN and AK treatments in brown rice liquid *Nuruk* at 24 hours after incubation, as the degree of rice-polishing increased, the activity of α-amylase was significantly lower (p<0.05). When brown rice was incubated in AN, it showed 8.83 units/mL at 48 hours after incubation, which was the highest activity, but there was no significantly difference (p<0.05), as the degree of rice- polishing was higher, the activity of α-amylase was lower. The activity of glucoamylase depending on the degree of rice-polishing showed 3,013 units/mL in AO treatment in brown rice liquid *Nuruk* at 24 hours after incubation, and the enzymatic activity was significantly higher (p<0.05). As the degree of rice-polishing increased, the activity of glucoamylase decreased, so liquid brown rice *Nuruk* showed the highest enzymatic activity, liquid white rice *Nuruk* was the lowest enzymatic activity. The highest enzymatic activity appeared in liquid *Nuruk* with brown rice at 48 hours after incubation. The activity of α-amylase, glucoamylase showed higher enzymatic productivity as the degree of rice-polishing was lower, and there was an inverse correlation with the total sugar content.

Keywords: enzymatic activity, liquid *nuruk*, degree of rice-polishing, yeast molds, *nuruk*

INTRODUCTION

There are *Aspergillus kawachii*, *Aspergillus niger, Aspergillus oryzae, Manascus kaoliang*, etc as molds in traditional *Nuruk* (Jung, 2012). The most isolated fungi from *Nuruk* are *Rhizopus oryzae* and *Aspergillus oryzae* which represent *Nuruk* (Ribes, Vanover-Sams, & Baker, 2000), *Aspergillus kawachii* is widely used for making *Soju*, and these fungi secrete various amylase enzymes such as α-amylase, glucoamylase, etc and saccharify starch.

There are a solid cultivation method in which *Nuruk* molds are inoculated on a raw material after cereals and the like are treated, and a liquid cultivation method in which *Nuruk* molds are inoculated by preparing a liquid medium by adding other nutrients to water in the recipe for preparing *Nuruk*. The solid cultivation method can produce various kinds of enzymes in a large amount, but it's difficult to control the temperature and humidity since the raw material is solid, and there is a risk of germ pollution because it's cultivated in semi-open form. Since microorganisms are naturally sessile and grows in making *Nuruk*, it's easy to inhibit the growth of the useful strain or to cause the deterioration of *Nuruk* (Kim et al., 2012), the complexity of the growth process and the contamination of harmful microorganisms have been reported (Kang, Park, & Jung, 1997, Kwon & Chun, 1988, Lee, Park, & Kung 1984). In addition, it's also difficult to control the quality of *Nuruk* and maintain enzymes (Lee, 1969). On the contrary, the liquid cultivation method is relatively safe for the propagation of all kinds of bacteria, and has advantages of simplification of making facilities and easy control of temperature and humidity uniformly. The quality characteristics of wheat *Nuruk* using *Rhizous oryzae* and *Aspergillus fungi* have been reported in the study of liquid *Nuruk* (Choi et al., 2011).

In this study, liquid *Nuruk* was prepared by the liquid culti-

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vation method, the activity of α-amylase and glucoamylase were measured depending on the degree of polishing rice as a medium and the kind of *Nuruk* molds, and the relationship between the enzymatic activity and the total sugar content of liquid *Nuruk* was investigated.

MATERIALS AND METHODS

Experimental Materials

Nuruk molds used in this experiment are *Aspergillus kawachii* (KCCM 11460), *Aspergillus niger* (KCCM 60315), *Aspergillus oryzae* (KCCM 11372), *Monascus kaoliang* (KCCM 60154) cultured in the Korean Microbiological Center, for *Nuruk* liquid, *Nuruk* provided from Yesan Traditional *Nuruk* Institute was suspended in an aqueous solution and *Nuruk* molds isolated from the suspension were used. The cultured *Nuruk* molds were used after incubation for 5 days in a thermostat at 30℃ utilizing PDA (potato dextrose agar) medium. The preparation of liquid *Nuruk* is as shown in Table 1. Liquid *Nuruk* was incubated by modifying Choi et al. (2012) method. Based on the yield, brown rice (100%), 3 Bun Domi (97.4% milling rice yields from brown rice), 7 Bun Domi (94.4%

Table 1. Mixture ratio of liquid *nuruk* by different kinds of microorganisms and degree of rice-polishing

Samples[1)]	Ingredients						
	A[2)] (g)	B[3)] (g)	C[4)] (g)	D[5)] (g)	Water (g)	LK[6)] (mL)	T[7)] (℃)
NK	100				300	15	25
		100			300	15	25
			100		300	15	25
				100	300	15	25
AK	100				300	15	25
		100			300	15	25
			100		300	15	25
				100	300	15	25
AN	100				300	15	25
		100			300	15	25
			100		300	15	25
				100	300	15	25
AO	100				300	15	25
		100			300	15	25
			100		300	15	25
				100	300	15	25
MK	100				300	15	25
		100			300	15	25
			100		300	15	25
				100	300	15	25

[1)] NK: *Nuruk*, AK: *Aspergillus kawachii*, AN: *Aspergillus niger*, AO: *Aspergillus oryzae*, MK: *Monascus kaoliang*.
[2)] A: Brown rice.
[3)] B: 97.4% milling rice yields from Brown rice.
[4)] C: 94.4% milling rice yields from Brown rice.
[5)] D: 92.0% milling rice yields from Brown rice.
[6)] LK: Liquid cultivation of *nuruk* (*nuruk* 15 g + water 30 g) and koji
[7)] T: Temperature.

milling rice yields from brown rice), 10 Bun Domi (92.0% milling rice yields from brown rice) were done and then they were washed and put in water for 1 hour, after draining them for 30 minutes, they were put in 1,000 mL flask by each 100g, in 500 mL erlenmeyer flask by each 300g, after sterilization and cooling under the conditions at 121℃ for 15 minutes, the suspensions of NK, AK, AN, AO, MK were inoculated so that the number of spores may be 1×10^5/mL. They were incubated at a culture temperature 30℃ at a churn speed of 200 rpm for 48 hours.

Measurement of the Activity of α-Amylase

For the activity of α-amylase in liquid *Nuruk*, as Mcllvaine buffer solution, the citric acid solution was titrated to pH 6.0 ~7.0 to 0.1N Na_2HPO_4 and used. As the iodine solution, 0.2 g of iodine and 2 g of potassium iodide were dissolved in 100 mL of 1N hydrochloric acid solution. 0.05 mL of the sample was diluted 200 fold with 10 mL of distilled water. After adding 5 mL of 1% soluble starch solution and 13 mL of Mcllvaine buffer solution to 1 mL of the diluted sample, 1 mL of 0.1% calcium chloride solution was added, and the mixture was reacted at 37℃ for 30 minutes. 0.2 mL was taken, 10 mL of iodine TS was added, and the absorbance was measured at 600 nm. To measure the blank, add 5 mL of 1% soluble starch solution and 13 mL of Mclvaine buffer solution to 200-fold diluted sample and then 1 mL of 0.1% calcium chloride solution. After making it reacted at 100℃ for 30 minutes, the absorbance was measured at 600 nm. α-amylase ultrapure was used as a standard curve and the content of α-amylase in the sample was determined.

The activity values of α-amylase were calculated using the following formula.

$$\text{Units/mg} = \frac{D_0 - D}{D_0} \times 10$$

D_0: The absorbance of control solution

D: The absorbance of solution after enzyme reaction

Measurement of the Activity of Glucoamylase

As for the activity of glucoamylase in liquid *Nuruk*, samples cultured at 30℃ were collected and used as samples for enzymatic activity measurement. The activity of glucoamylase was measured using the saccharification force fractionation kit of glucose C II-kit (Wako Pure Chemical Industries, Osaka Japan), the reagent for measuring the activity of glucoamylase was prepared as follows.

A. 0.1 mol/L acetate buffer (pH 4.5) solution
B. As substrate solution, add 40 mL of distilled water to 1g of water soluble starch, dissolve it by heating, cool, add 50 mL of A solution, and distilled water to make 100 mL
C. 0.05 mol/L acetate buffer (pH 5.5)
D. 0.6 mol/L NaOH solution
E. Enzyme solution: take 100 mg of glucoamylase precisely, add 1 mL of ion-exchanged water, add 50 μL of this solution and add 50 mL of C solution
F. Coloring solution: Chromogen solution (glucose C II-Kit, Wako)
G. 2 g/L of glucose standard solution

Take 2 mL of B solution in a beaker and preheat it at 37℃ for 5 minutes, add 0.5 mL of E solution and preheat it at 37℃ for 15 minutes. The solution obtained by adding 0.5 mL of D solution was used as a' solution, the solution of adding 2 mL of B solution, 0.5 mL of D solution, and 0.5 mL of E solution to the beaker was used b' solution. For the test sample solution, take 3 mL of F solution and preheat it at 37℃ for 5 minutes and then add 20 μL of b' solution and treat it at 37℃ for 5 minutes. In addition, Blank 1 was prepared by pre-heating it at 37℃ for 5 minutes after taking 3 mL of F solution, adding 20 μL to b' solution and reacting at 37℃ for 5 minutes. Blank 2 was prepared by taking 3 mL of F solution, pre-heating it at 37℃ for 5 minutes, adding 20 μL of distilled water, and reacting at 37℃ for 5 minutes. For the standard solution, take 3 mL of F solution, pre-heat it at 37℃ for 5 minutes, add 20 μL of G solution and react it at 37℃ for 5 minutes.

Blank 1, the sample solution prepared in the above and the standard solution were used for measurement by making Blank 2 a control solution. The absorbance was measured at 505 nm using UV/Vis spectrophotometer (JASCO, V-550) and calculated by the following equation. Glucoamylase 1

units was defined as the amount of enzyme that produced 1 μmol of glucose for 1 minute at pH 4.5, 37℃.

$$\text{Units/mg} = 2\times\frac{E_1-E_0}{E_2}\times\frac{10^3}{180.6}\times3.0\times\frac{1}{15}\times\frac{1}{0.5}\times\frac{1}{S}\times1{,}000$$

E_1: Absorbance of the sample solution
E_0: Absorbance of Blank 1
E_2: Absorbance of the standard solution
3.0: Total solution amount
15: Reaction time (min)
0.5: Amount of E solution
180.16: Molecular weight of glucose
S: Amount of taking glucoamylase

Measurement of the Total Sugar Content

For the phenol-sulfuric acid method, the total sugar content was measured with Kang KH et al. (1998). 2 mL of the sample was put in a test tube and 1 mL of 5% (v/v) phenol reagent (Shinyo Pure Chemical Co. Ltd., Osaka, Japan) solution was added. After adding 5 mL of 95% sulfuric acid (Deajung Chemical & Metals Co. Korea), the mixture was allowed to stand at room temperature for 30 minutes and then the absorbance was measured at 470 nm using UV/Vis spectrophotometer (JASCO. V-550). The sugar content (mg/mL) was determined by comparing with the standard curve prepared by the above method using glucose as a standard substance.

Statistical Analysis

Data were subjected to analysis of variance (ANOVA) and differences between means at $p<0.05$ were analyzed using the Tukey test. The statistics package version 4.0 (Analytical Software, Tucson AZ, USA) was used for statistical analysis. All chemical measurements were replicated three times unless mentioned otherwise and the average values were reported.

RESULTS AND DISCUSSION

Nuruk and *Nuruk* molds were cultured in a liquid medium and then the activity of α-amylase, glucoamylase and the total sugar content were measured by adding NK, AK, AN, AO, MK to investigate the relationship between the enzymatic activity and sugar of liquid *Nuruk* depending on the degree of rice-polishing

Changes in α-Amylase Activity

Table 2 shows the results of α-amylase activity depending on the degree of rice-polishing and the types of *Nuruk* and *Nuruk* molds. The activity of each enzyme was different. The enzymatic activity of *Nuruk* is very important in the role of liquefying and glycation power. The activity of α-amylase

Table 2. Change in α-amylase activity of according to types of *nuruk* molds, degree of rice-polishing and cultivation time

Time (hrs)	Samples[1)]	α-Amylase activity (units/mL)				
		NK[2)]	AK[3)]	AN[4)]	AO[5)]	MK[6)]
24	A	4.43±0.13^{bc}[7)]	8.72±0.03^{ab}	8.82±0.03^{ab}	8.00±0.03^{a}	5.11±0.01^{a}
	B	4.10±0.02^{d}	8.72±0.02^{ab}	8.75±0.03^{ab}	7.25±0.10^{c}	4.82±0.02^{b}
	C	4.22±0.11^{cd}	8.66±0.01^{bc}	8.77±0.04^{ab}	5.90±0.02^{d}	1.76±0.08^{g}
	D	3.79±0.08^{e}	8.58±0.04^{d}	8.73±0.03^{b}	4.23±0.03^{g}	1.91±0.07^{f}
48	A	4.95±0.03^{a}	8.73±0.03^{a}	8.83±0.01^{a}	8.16±0.01^{a}	3.73±0.03^{c}
	B	4.53±0.12^{b}	8.72±0.02^{ab}	8.78±0.08^{ab}	7.65±0.03^{b}	3.73±0.08^{c}
	C	4.33±0.03^{bcd}	8.63±0.02^{cd}	8.77±0.02^{ab}	4.63±0.11^{f}	2.11±0.04^{e}
	D	4.14±0.11^{d}	8.59±0.01^{d}	8.73±0.02^{ab}	5.07±0.08^{e}	2.43±0.06^{d}

[1)] A: Brown rice, B: 97.4% milling rice yields from brown rice, C: 94.4% milling rice yields from brown rice, D: 92.0% milling rice yields from brown rice; [2)] NK: *Nuruk*; [3)] AK: *Aspergillus kawachii*; [4)] AN: *Aspergillus niger*; [5)] AO: *Aspergillus oryzae*; [6)] MK: *Monascus kaoliang*; [7)] Quoted values are means±standard deviations of triplicate measurements. The values with different letters in the same column are significantly different ($p<0.05$).

depending on the types of *Nuruk* molds was shown to be 8.82 and 8.72 units/mL, respectively, which was highest in AN and AK treatments in liquid brown rice *Nuruk* at 24 hours after the incubation, followed by AO treatment at 8.00 units/mL. The activity of α-amylase according to the degree of rice-polishing was 8.82~8.58 units/mL in AN treatment, the enzymatic activity depending to the degree of rice-polishing was 8.72~8.58 units/mL in AK treatment so there was a significant difference by the degree of rice-polishing (p<0.05). For AO treatment, as the degree of rice-polishing was higher, the activity was lower so it showed 8.72~8.58 units/mL. *Nuruk* treatment was not significantly changed from 4.43 to 3.79 units/mL, the activity of α-amylase tended to be lower by 5.11 ~1.91 units/mL in MK treatment as the degree of rice-polishing was higher.

The results of 48 hours were similar to those of 24 hours after culture. The highest activity was 8.83 units/mL in AN treatment of liquid brown rice *Nuruk*, 8.73 units/mL in AK treatment. AO treatment was 8.16 units/mL, which was slightly higher than at 24 hours. AN treatment was 8.83~8.73 units/mL for 48 hours culture, indicating that the rate of α-amylase activity did not differ significantly (p<0.05). AO treatment was 8.16~5.07 units/mL, and the activity of α-amylase tended to decrease as the degree of rice-polishing was higher. *Nuruk* treatment also showed a tendency to lower the activity of α-amylase as the degree of rice-polishing increased from 4.95~4.14 units/mL. This suggests that the content of the total sugar isolated from rice in liquid culture increase as the degree of rice-polishing increases.

Regarding the activity of α-amylase according to the degree of rice-polishing, the activity of A sample cultured for 48 hours was significantly higher than that of all samples (p<0.05).

Changes of Glucoamylase Activity

Table 3 shows the results of glucoamylase activity according to the degree of rice-polishing and the types of *Nuruk* and *Nuruk* molds. The activity of glucoamylase depending on the types of molds and degree of rice-polishing was found to be higher in liquid brown rice *Nuruk* at 24 hours after incubation in the order of AO treatment > NK treatment > MK treatment > AN treatment > AK treatment, AO treatment showed the highest activity by 3,013 units/mL. It was found that the enzymatic activity varied depending on the degree of rice-polishing with each sample in the preparation of liquid *Nuruk* using AO fungi. In the NK treatment, liquid brown rice *Nuruk* showed 2,665 units/mL but liquid white rice *Nuruk* decreased to 1,197 units/mL. In MK treatment, liquid brown rice *Nuruk* was 1,781 units/mL and liquid white rice *Nuruk* was 216 units/mL, and its activity was lower than that of liquid brown rice *Nuruk* and AN activity tended to decrease depending on the degree of rice-polishing.

AO treatment at 48 hours after incubation showed the

Table 3. Change in glucoamylase activity of according to types of *nuruk* molds, degree of rice-polishing and cultivation time

Time (hrs)	Samples[1)]	Glucoamylase activity (units/mL)				
		NK[2)]	AK[3)]	AN[4)]	AO[5)]	MK[6)]
24	A	2,665±2.21[a7)]	1,111±0.12[d]	1,120±0.02[b]	3,013±0.10[a]	1,781±0.31[a]
	B	2,351±1.89[c]	1,002±0.08[e]	210±1.18[h]	2,763±0.32[b]	694±1.32[d]
	C	1,904±1.20[f]	842±1.68[f]	406±1.34[be]	2,256±0.13[d]	160±1.19[h]
	D	1,197±1.58[h]	752±0.77[h]	217±0.68[g]	2,263±0.23[c]	216±0.32[e]
48	A	2,551±1.32[b]	1,264±0.08[a]	1,472±1.18[a]	3,048±0.02[h]	1,477±0.32[b]
	B	2,272±2.56[d]	1,201±0.77[c]	954±0.12[e]	2,132±1.18[g]	767±0.33[c]
	C	2,135±0.51[e]	1,206±1.18[b]	483±0.03[d]	2,209±0.06[f]	189±1.69[g]
	D	1,645±0.32[g]	761±0.31[g]	224±0.78[f]	1,423±1.21[e]	206±0.78[f]

[1)] A: Brown rice, B: 97.4% milling rice yields from brown rice, C: 94.4% milling rice yields from brown rice, D: 92.0% milling rice yields from brown rice; [2)] NK: *Nuruk*, [3)] AK: *Aspergillus kawachii,* [4)] AN: *Aspergillus niger,* [5)] AO: *Aspergillus oryzae*, [6)] MK: *Monascus kaoliang*, [7)] Quoted values are means±standard deviations of triplicate measurements. The values with different letters in the same column are significantly different (p<0.05).

highest enzymatic activity in liquid *Nuruk* with brown rice by 3,048 units/mL, as the degree of rice-polishing was higher, the enzymatic activity decreased significantly (p<0.05) so the enzymatic activity of liquid white rice *Nuruk* was significantly lowered to 1,423 units/mL. In most experiment treatments, the enzymatic activity tended to decrease according to the degree of rice-polishing, in AO treatment, liquid white rice *Nuruk* was 1,423 units/mL, which was significantly decreased at 24 hours after incubation.

Total Sugar Content and Enzymatic Activity

Table 4 shows the total sugar content depending on the degree of rice-polishing and the types of *Nuruk* and *Nuruk* molds. In the case of the liquid cultivation for *Nuruk* molds, the activity of α-amylase and glucoamylase were closely related to sugar. α-amylase, glucoamylase showed higher enzymatic activity as the degree of rice-polishing was lower, indicating the best enzymatic activity in liquid brown rice *Nuruk*. In addition, the total sugar content shown in Table 4 showed a negative correlation (Ruiter, & Visser, 1997; Sugimoto et al., 2011). It's desirable to control the total sugar content in the production of glucoamylase by cultivation of *Aspergillus niger* (Guido et al., 2007; VanKuyk et al., 2008), it's considered that the total sugar concentration of the medium is controlled to be high enough to produce α-amylase, glucoamylase when cultured in brown rice (Sudo et al., 1993; Sudo et al., 1995).

The difference in the productivity of α-amylase, glucoamylase depending on the degree of rice-polishing is because the total sugar content varies according to the physical state of the carbon source used in the culture. As shown in Table 3 and 4, the brown rice used for the medium as a carbon source exhibiting high enzyme productivity in the liquid cultivation of *Nuruk* molds is covered with the envelope, and it's considered that the culture is proceeding with inhibiting the excessive elution of the starch into the medium. Also, the difference in enzymatic activity depending on the degree of rice-polishing can be applied to a general grain material as well as a new methodology established in liquid cultivation. Liquid *Nuruk* using brown rice was found to enable high production of starch degrading enzyme.

CONCLUSION

The results of measuring the activity of α-amylase, glucoamylase in liquid *Nuruk* prepared using NK, AK, AN, AO and MK were as follows to investigate the relationship between the enzymatic activity and the total sugar content of liquid *Nuruk* depending on the degree of rice-polishing.

The activity of α-amylase by the types of *Nuruk* molds was found to be 8.82, 8.72 units/mL, respectively in AN and AK treatments in liquid brown rice *Nuruk* at 24 hours after incubation, the activity of α-amylase was decreased as the degree

Table 4. Change in total sugar of according to types of *nuruk* molds, degree of rice-polishing and cultivation time

Time (hrs)	Samples[1)]	Total sugar (mg/mL)				
		NK[2)]	AK[3)]	AN[4)]	AO[5)]	MK[6)]
24	A	1.90±0.02^{d}	0.74±0.02^{f}	0.25±0.02^{d}	0.17±0.00^{e}	0.57±0.02^{f}
	B	2.40±0.02^{c}	1.10±0.03^{e}	0.33±0.02^{d}	0.26±0.04^{d}	1.31±0.01^{d}
	C	2.35±0.03^{c}	1.68±0.05^{bc}	0.23±0.10^{d}	0.85±0.01^{c}	1.56±0.08^{c}
	D	3.25±0.04^{a}	1.37±0.00^{d}	0.96±0.05^{b}	0.86±0.05^{c}	1.82±0.01^{b}
48	A	1.66±0.11^{e}	1.55±0.01^{c}	0.54±0.01^{c}	0.23±0.01^{de}	1.18±0.03^{e}
	B	1.50±0.03^{f}	1.86±0.08^{a}	0.53±0.01^{c}	0.17±0.02^{e}	1.54±0.02^{c}
	C	1.95±0.05^{d}	1.53±0.12^{cd}	1.24±0.03^{a}	1.02±0.02^{b}	1.57±0.02^{c}
	D	2.60±0.02^{b}	1.82±0.07^{ab}	1.35±0.02^{a}	1.66±0.02^{a}	2.06±0.03^{a}

[1)] A: Brown rice, B: 97.4% milling rice yields from brown rice, C: 94.4% milling rice yields from brown rice, D: 92.0% milling rice yields from brown rice. [2)] NK: *Nuruk,* [3)] AK: *Aspergillus kawachii*, [4)] AN: *Aspergillus niger*, [5)] AO: *Aspergillus oryzae*, [6)] MK: *Monascus kaoliang*, [7)] Quoted values are means±standard deviations of triplicate measurements. The values with different letters in the same column are significantly different(p<0.05).

of rice-polishing increased, the enzymatic activity of AK treatment was 8.72~8.58 units/mL, which was significantly different according to the degree of rice-polishing (p<0.05). When brown rice was cultured with AN for 48 hours, it was the highest activity by 8.83 units/mL, the activity of α-amylase tended to decrease as the degree of rice-polishing was higher.

The activity of glucoamylase depending on the degree of rice-polishing was significantly higher in the order of AO treatment > NK treatment > MK treatment > AN treatment > AK treatment at 24 hours after incubation, AO treatment showed high enzymatic activity by 3,013 units/mL. As the degree of rice-polishing was higher, the activity of glucoamylase decreased so liquid brown rice *Nuruk* showed the highest enzymatic activity and liquid white rice *Nuruk* showed the lowest enzymatic activity. At 48 hours after incubation, AO treatment showed the highest enzymatic activity in liquid *Nuruk* with brown rice, as the degree of rice-polishing was higher, the enzymatic activity decreased significantly (p<0.05) so the enzymetic activity of liquid white rice *Nuruk* was signifycantly lower by 1,423 units/mL (p<0.05). The highest enzymatic activity was observed in liquid *Nuruk* added with brown rice.

The activity of α-amylase, glucoamylase showed higher enzyme productivity as the degree of rice-polishing was lower, and showed an inverse correlation with the total sugar content. Liquid brown rice *Nuruk* showed the best enzyme productivity, and it was desirable to control the total sugar concentration to a low level for the production of α-amylase, glucoamylase in liquid culture.

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