การเพิ่มระดับของโปรตีนของมันสำปะหลังโดยใช้ยีสต์ในกระบวนการหมัก

Increasing protein content of cassava (*Manihot esculenta*, Crantz) using yeast in fermentation

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บทคัดย่อ: วัตถุประสงค์ของงานทดลองครั้งนี้เพื่อศึกษาการเจริญเติบโตของยีสต์ *Saccharomyces cerevisiaes* ในอาหารเลี้ยงเชื้อที่แตกต่างกันและศึกษาถึงการเพิ่มโภชนะของมันเส้นโดยหมักร่วมกับยีสต์ งานทดลองครั้งนี้ใช้แผนการทดลองแบบ 2x4 Factorial arrangement in CRD โดยศึกษาถึงการเจริญเติบโตของยีสต์ *Saccharomyces cerevisiae* จำนวน 2 ชนิด คือ 1. Baker's yeast (YB), 2. Brewer's yeast (YC) โดยทำการเพาะเลี้ยงในอาหารเลี้ยงเชื้อจำนวน 4 ชนิด ตามสัดส่วนของ ยูเรีย:กากน้ำตาล:น้ำ ดังนี้ 40:32:100 (M1), 48:24:100 (M2), 56:16:100 (M3), 64:08:100 (M4) ค่า pH ที่ระดับ 3.5-5.0 ในอุณหภูมิห้อง ทำการศึกษาเลี้ยงเชื้อเป็นระยะเวลา 120 ชม. ในระหว่างเลี้ยงเชื้อทำการเก็บตัวอย่างที่ 0 และทุกๆ 6 ชั่วโมงของการเลี้ยงเชื้อเพื่อวัดค่าการดูดกลืนแสงที่ 600 nm หลังจากนั้นนำน้ำหมักยีสต์ทั้ง 8 ทรีทเมนต์ ที่ระยะเวลาที่บ่มที่เหมาะสมที่สุด (66 h) มาหมักร่วมกับมันเส้นในสัดส่วน 1 ml : 1.3 g ซึ่ง สำหรับมันเส้นหมักยีสต์ (YEFECAP) นำไปทำการวิเคราะห์องค์ประกอบทางเคมี จากการทดลองครั้งนี้พบว่า การเจริญเติบโตของ S. *cerevisiaes* ใน YBM3 (baker's yeast + urea: molasses: water ในสัดส่วน 56:16:100 (M3)) มีปริมาณยีสต์สูงสุด (30x1011 cell/ml) ที่ 66 ชม. ของการเลี้ยงเชื้อ ในส่วนของ YEFECAP พบว่า YBM4 (baker's yeast + urea:molasses:water ในสัดส่วน 64:08:100 (M4) มีองค์ประกอบของโปรตีนหยาบสูงสุดที่ 47.5% มากไปกว่านั้นยังต้องการการศึกษาเพิ่มเติมถึงการใช้ YEFEECAP เป็นแหล่งโปรตีนทดแทนกากถั่วเหลืองในสูตรอาหารข้นในตัวสัตว์ โดยเฉพาะอย่างยิ่งในสัตว์ที่กำลังให้ผลผลิตต่อไป

คำสำคัญ: *Saccharomyces cerevisiae*, Baker's yeast, Brewer's yeast, มันเส้นหมักยีสต์, โปรตีน

ABSTRACT: Aims of this experiment were to study kinetic of yeast *Saccharomyces Cerevisiaes* growth in different mediums and to study enrichment of cassava chip by fermentation using yeast. Experiments were assigned according to a 2x4 Factorial arrangement in CRD to study growth kinetic of 2 yeast were Baker's yeast (YB) and Brewer's yeast (YC) cultivated in 4 medium (urea:molass:water) = 40:32:100 (M1), 48:24:100 (M2), 56:16:100 (M3), 64:08:100 (M4) at pH 3.5-5.0 in room temperature for 120 h, kinetic of yeast growth were collected at 0 and every 6 h post-cultivation. All treatments were selected from the optimum cultivation time (66 h) mixed with cassava chip at ratio at 1 ml:1.3 g, For yeast fermented cassava chip protein (YEFECAP) products were analyzed for proximate composition. The results show that kinetic growth of *S. cerevisiae* in YBM3 (Baker's yeast with urea: molasses: water at ratio 56:16:100 (M3)) was highest in number (3.0×10^{11} cell/ml) at 66 h post-cultivation. However, YEFECAP production in YBM4 (Baker's yeast with urea: molasses: water at ratio 64:08:100 (M4)) was highest in protein content at 47.5% CP. Furthermore, further research is required to study the use of YEFECAP as a protein source to replace soybean meal in *in vivo* feeding trials especially in productive ruminants.

Keyword: *Saccharomyces cerevisiae*, Baker's yeast, Brewer's yeast, Yeast fermented cassava chip protein (YEFECAP), Protein

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Introduction

Eukaryotic organisms can be considered for high protein production performance. The two chief strategies with regards to substrate to consider are low grade waste material, or to use relatively simple carbohydrate source to produce microbial material containing very high quality of protein. Varying concentration of urea were added as nitrogen supplement for yeast growth, urea is a low cost fertilizer to support maximum microbial biomass protein production. Furthermore, molasses contains readily utilizable carbohydrates available in the form of fermentable sugars can be used for yeast growth (Polyorach et al., 2011). Process of protein enrichment of animal feed using the microorganism to improve the nutritional value of cassava has been evaluating. Recently, Oboh and Akindahinsi (2003) reported that *S. cerevisae* could also be used for enriching cassava products. Boonnop et al. (2009) demonstrated that supplementation of cassava chip with Baker's yeast (*S. cerevisae*) could be increased in crude protein from 2% to 32.4%. Moreover, Brewer's yeast (*S. cerevisae*) is a by-product that can be produced in association with the production of beer, one of interesting microorganism used for enrichment of animal feed.

Therefore, the objectives of this study were to study kinetic of yeast *S. cerevisiaes* (Baker's yeast and Brewer's yeast) growth in different mediums and to study enrichment of cassava chip by fermentation using Baker's yeast and Brewer's yeast.

Materials and Methods

Treatments and experimental design: Eight treatments combination were randomly assigned according to a 2x4 Factorial arrangement in a Completely randomized design (CRD) to study growth kinetic of two yeast type (*S. cerevisiaes*); 1. Baker's yeast (YB), 2. Brewer's yeast (YC) cultivated in four medium (urea:molass:water) (g/g/ml) = 40:32:100 (M1), 48:24:100 (M2), 56:16:100 (M3), 64:08:100 (M4) as treatment combinations were YBM1=YB+M1, YBM2=YB+M2, YBM3=YB+M3, YBM4=YB+M4, YCM1=YC+M1, YCM2=YC+M2, YCM3=YC+M3, YCM4=YC+M4. **Yeast cultivation:** Activated yeast: by weigh 20 g of yeast into a flask and add 20 g sugar and 100 ml distilled water, mixed well and incubate at room temperature for 1 h (A). Liquid media preparation: by weigh and mix well 24 g molasses, 100 ml distilled water, 48 g urea and then adjust pH of medium solution using H_2SO_4 to achieve final pH 3.5-5 (B). Mix (A) and (B) at 1:1 ratio then flush with air for 120 h at room temperature by using air pump (600 W). Yeast fermented liquid was sampling at 0 and every 6 h post-cultivation. **Yeast fermented cassava chip protein (YEFECAP) production:** Should the best time (66 h) of cultivation, transfer yeast media solution mixed with cassava chip ratio at 1 ml : 1.3 g, then dry under shade for 72 h, and followed by sun-drying for 48 h. Final products were stored in plastic bag for mixing in the concentrate. **Determination of yeast growth:** The growth of yeast were measured absorbance using spectrophotometer was utilized to measure the absorbance of light. The absorbance of a yeast culture was a measurement device to determine the amount of yeast cells presented in liquid

culture. The more cells in a culture involve the more density. A sample to appropriate concentrations as needed was measured the absorbance of the sample with the spectrophotometer at 600 nm (Bausch and Lomb spectrophotometer, VWR Scientific Inc. N.Y.). For the culture sample, a good absorbance value should yield linear relationship between the number of cells and the absorbance. Therefore, to produce a calibration curve of the relation between the absorbance and cell amounts was examined by an equipment for counting the number of cells in a measured volume namely Hemacytometer. Consequently, the relation between absorbance values and cell amounts can be utilized to calibrate the number of the yeast in the experiment. **Chemical analysis:** Dry matter (DM) of yeast fermented cassava chip protein (YEFECAP) at different level of liquid media were analyzed by drying at 100 °C for 12 h in a hot air oven, ash, EE, CP, NDF, ADF determined according standard methods. **Statistical analysis:** All data were statistically analyzed using analysis of variance of a Completely randomized design with a 2x4Factorial arrangement using Proc. GLM procedure (SAS, 1998). Treatment means were statistically compared using Duncan's New Multiple Range Test (Steel and Torrie, 1980).

Results and Discussions

The standard curves were produced from 8 treatment combinations at 0 h. The relationship between absorbance values and number of cells was found that, when increasing concentration of yeast absorbance value and number of cells were increased, the linear relation ship between cell numbers and absorbance. The values of r^2 of YBM1, YBM2, YBM3, YBM4, YCM1, YCM2, YCM3 and YCM4 were 0.92, 0.97, 0.96, 0.98, 0.92, 0.98, 0.98 and 0.94, respectively. Therefore, the regression equations between spectrophotometer and direct counts from standard curves could be used in this experiment.

Baker's yeast and Brewer's yeast were cultured in plastic bucket containing 5000 ml of yeast fermented liquid at pH between 3.5 to 5.0 and room temperature (**Figure 1**). It was shown that most of the treatments resulted in highest growth starting from 60 h post-cultivation except for YBM3 and YCM3 started from 66 h post-cultivation. At 66 h post-cultivation YBM3 was highest in number at $3x10^{11}$cell/ml while YBM1 and YCM1 were lowest on number $2.1x10^{11}$cell/ml. The pH and temperature in this study were similar with previous work, the optimum pH levels for *S. cerevisae* cultivation were from 3.5 to 6.0 and temperature levels were from 20 to 40 °C and optimal cultivation time between 60 to 80 h by highest biomass (Asli, 2010).

Figure 2, shows the growth curve of yeast *S. cerevisiae* (Baker's yeast and Brewer's yeast) in medium M1, M2, M3 and M4 (urea:molasses:water; M1=40:32:100, M2=48:24:100, M3=56:16:100 and M4=64:08:100). It appeared that significant differences ($P<0.05$) at 42, 54, 60, 66, 72, 78, 84, 90, 96, 102, 108, 114 and 120 h post-cultivation by medium M1, M2 and M3 when increasing proportion of urea, yeast growth were higher and highest in medium M3, while in medium M4 resulted in a poor result. Effect of yeast type on kinetic of yeast growth was shown in Figure 3. It was found that growth of Baker's yeast (YB) were significant higher than Brewer's yeast (YC) ($P<0.05$), especially at 18, 24, 30, 90, 102, 108 and 114 h post-cultivation.

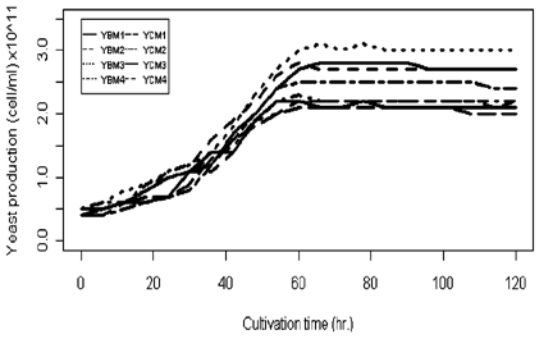

Figure 1 Effects of different yeast type anddifferent medium on kinetic of yeast growth.

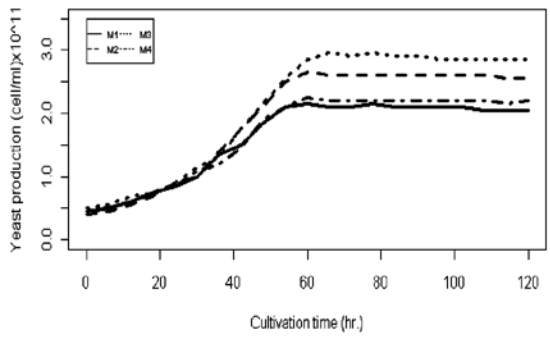

Figure 2 Effects of medium on kinetic of yeast growth.

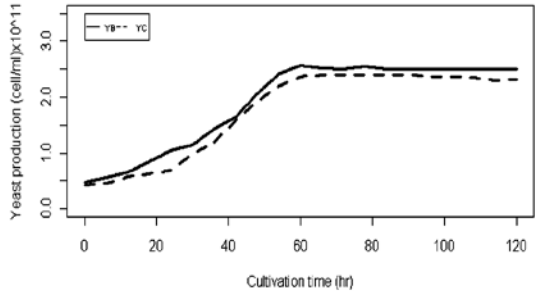

Figure 3 Effects of yeast type on kinetic of yeast growth.

Effects of different yeast type and medium on chemical composition of yeast fermented cassava chip protein (YEFECAP) product are shown in Table 1. It was found that interaction between yeast type and medium were significantly ($p<0.01$) differenced on percentage of DM, OM, CP and ADF. In addition of YBM1 ha highest DM (92.1%) while YCM1 and YCM2 were lowest (90.1%). Moreover, OM of YBM3 was highest (97.3%) whereas YCM4 was lowest (96.0%). The crud protein of YEFECAP products in YBM4 was highest follow by YBM3, YCM4, YBM2, YCM3, YCM2, YBM1 and YCM1, values were 47.5, 46.4, 41.9, 40.6, 38.5, 37.4, 33.7 and 29.3 respectively. However, there were no changes in EE and NDF contents of the YEFECAP products. The protein contents of product were higher than that reported by Boonnop et al. (2009) and Wanapat et al. (2011), it could be due to particle size of cassava chip was smaller and yeast fermented liquid and cassava ratio was higher than those reports.

Table 1 Chemical composition of YEFECAP (% of DM).

	YB				YC					Contrast[1]		
Item	M1	M2	M3	M4	M1	M2	M3	M4	SEM	A	B	A*B
DM	92.1^{a}	90.9^{b}	90.6^{bc}	90.6^{bc}	90.1^{c}	90.1^{c}	90.3^{c}	91.9^{b}	0.16	**	**	**
OM	96.7^{cd}	96.9^{c}	97.3^{a}	97.2^{ab}	97.2^{ab}	97.1^{b}	96.6^{d}	96.0^{e}	0.05	**	**	**
CP	33.7^{g}	40.6^{d}	46.4^{b}	47.5^{a}	29.3^{h}	37.4^{f}	38.5^{e}	41.9^{c}	0.28	**	**	**
EE	7.3	8.7	9.9	7.9	8.4	9.6	9.7	8.7	0.69	ns	ns	ns
NDF	6.9	6.8	6.7	6.1	6.6	6.5	6.0	6.4	0.16	ns	ns	ns
ADF	4.5^{c}	4.1^{e}	3.5^{f}	4.3^{d}	5.3^{a}	4.9^{b}	4.0^{e}	4.5^{c}	0.06	**	**	**

^{a,b,c,d,e,f,g,h} Means with different superscripts differ (P<0.05), [1] A=effect of yeast, B=effect of medium, A*B= interaction between yeast and medium, YB= Baker's yeast, YC= Brewer's yeast, M1= 40: 32: 100 (urea: molasses: water), M2= 48: 24: 100, M3= 56: 16: 100, M4= 64: 08: 100, SEM= Standard error of the mean, ns= Non-significant difference, **P<0.01.

Conclusion and Recommendations

Based on the results of this experiment, it could be concluded that kinetic growth of *Sacchromyces cerevisiae* in YBM3 (Baker's yeast with urea:molasses:water ratio at 56:16:100 (M3)) was highest in number ($3.0x10^{11}$ cell/ml) at 66 h of cultivation. However, YEFECAP products in YBM4 (Baker's yeast with urea: molasses: water ratio at 64:08:100 (M4)) was highest in protein content at 47.5% CP. However, further research is required to study the use of YEFECAP as a protein source to replace soybean meal in feeding trials in productive ruminants.

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References

Asli, M. S. 2010. A study on some efficient parameters in bach fermentation of ethanol using *Saccharomyces cerevesiae* SC1 extracted from fermented siahe sardasht pomace. Afr. J. Biotechnol. 9: 2906-2912.

Boonnop, K., M. Wanapat, N. Nontaso, and S. Wanapat. 2009. Enriching nutritive value of cassava root by yeast fermentation. Sci. Agric. (Piracicaba, Braz.). 66: 616-620.

Oboh, G., and A. A. Akindahinsi. 2003. Biochemical changes in cassava products (flour & gari) subjected to *Sacchromyces cerevisiae* solid media fermentation. Food Chem. 82: 599-602.

Polyorach, S., M. Wanapat, C. Wachirapakorn, C. Navanukroaw, S. Wanapat, and N. Nontaso. 2011. **Supplementation of yeast fermented liquid (YFL) and coconut oil on rumen fermentation characteristics, N-balance and urinary purine derivatives in beef cattle.** J. Anim. Vet. Adv. 10: 2084-2089.

SAS. 1998. SAS/STAT User's Guide. Version 6.12. SAS Inst. Inc., Cary, NC.

Steel, R. G. D., and J. H. Torrie. 1980. Principles and Procedures of Statistics: A Biometerial Approach, second ed. McGraw-Hill, New York, U.S.A.

Wanapat, M., S. Polyorach, V. Chanthakhoun and N. Sornsongnern. 2011. Yeast-fermented cassava chip protein (YEFECAP) concentrate for lactating dairy cows fed on urea-lime treated rice straw. Livest. Sci. 139: 258-263.