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Enrichment of protein content in cassava (*Manihot esculenta* Crantz) by supplementing with yeast for use as animal feed

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Abstract

The aim of this investigation was to study the kinetics of yeast *S. cerevisiae* growth in different media and to study the enrichment of cassava chip by fermentation using yeast. Experiment was conducted according to a 2x4 factorial arrangement in a Completely Randomized Design (CRD) to study growth kinetics of Baker's yeast (YB) and Brewer's yeast (YC) cultivated in 4 media with following composition: (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 at 27°C for 120 h. The kinetic data 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 of 1 ml:1.3 g. For yeast fermented cassava chip protein (YEFECAP) products were analyzed for proximate composition. The results showed that kinetic growth of *S. cerevisiae* in YBM3 was the 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% crude protein CP when compared to other treatments. 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.

Key words: *Saccharomyces cerevisiae*, Baker's yeast, Brewer's yeast, Yeast fermented cassava chip protein (YEFECAP), Protein enrichment, Cassava chip

Introduction

Cassava or tapioca (*Manihot esculenta*, Crantz) is an annual tropical tuber crop grown widely in tropical and sub-tropical countries (Wanapat, 2003). This plant is easily grown under minimal management and it adapts to poor soil conditions, low rainfall, high temperature and pest tolerance (Salami and Akintokun, 2008). Usually, cassava is grown for root production as energy sources. Cassava tuber can be processed into dried chip which consists of soluble carbohydrate 76-81%, but is low in crude protein (1-3% CP) depending on cultivar. Moreover, *S. cerevisiae* has been widely used for protein production (Lang et al., 1997).

Eukaryotic microorganisms can be considered a suitable host for the production because firstly, growth of microorganism is very much fast, secondly, a broader range of materials may be considered as suitable substrates depending on the microorganism chosen. The two chief strategies with regard 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 (Reed and Nagodawithana, 1995). Urea is a low cost fertilizer (Al-Moshileh et al., 2005), varying concentration of urea were added as nitrogen supplement for yeast growth. Ali et al. (2009) showed that urea could support maximum microbial biomass protein production. Furthermore, molasses contains readily utilizable carbohydrates available in the form of fermentable sugars and can be used for yeast growth (Mukhtar et al., 2010; Polyorach et al., 2011), almost 75% of the world's molasses come from sugarcane grown in tropical climates of Asia and South America (Piggot, 2005). The process of protein enrichment of animal feed using the microorganism to improve the nutritional value of cassava has been evaluated (Oboh, 2006). This method of upgrading the protein content of cassava has been developed. Recently, Oboh and

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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 increase crude protein from 2% to 32.4%. Moreover, Brewer's yeast (*S. cerevisae*) is 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 as follows: (a) To determine the optimal media for faster growth of yeast and (b) To determine which yeast-media combination would enhance crude protein content in cassava.

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 kinetics of two yeast type (*S. cerevisiaes*); 1. Baker's yeast (YB), 2. Brewer's yeast (YC) cultivated in four media with differing ratios of urea:molass:water (g/g/ml) = 40:32:100 (M1), 48:24:100 (M2), 56:16:100 (M3), 64:08:100 (M4).

Materials

The commercial Baker's yeast (*S. cerevisiae*) and Brewer's yeast (*S. cerevisiae*), manufactured by Berly Speciality Industries and by-product from Khon Kaen Brewery Co., Ltd. respectively were used in the fermentation processes. Cassava chip, commercial grade urea and sugar cane molasses were purchased from the local shop.

Yeast cultivation

Preparation of activated yeast: 20 g of Baker's yeast/ Brewer's yeast was weighed and put into a flask and added 20 g cane sugar and 100 ml distilled water, mixed well and incubated at room temperature for 1 h (A). Liquid media preparation: 24 g molasses was weighed and dissolved in 100 ml distilled water, followed by addition of 48 g urea and then adjusted pH of the solution using H_2SO_4 to achieve a final pH 3.5-5 (B). Mixed (A) and (B) at 1:1 ratio then flushed with air for 120 h at room temperature by using air pump (600 W). Yeast fermented liquid was sampled at 0, 6, 12, 18, 24, 30, 36, 42, 48, 54, 60, 66, 72, 78, 84, 90, 96, 102, 108, 114, 120 h post-cultivation.

Yeast fermented cassava chip protein (YEFECAP) production

Choosed the best time (66 h) of cultivation, transfer yeast media solution mixed with cassava chip ratio at 1 ml : 1.3 g, then dried 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 kinetics of yeasts were determined by using regression equation (Figure 1.) from standard curve between the absorbance (Ao) at 600 nm and number of cells (cell/ml) from concentration of Baker's yeast/Brewer's yeast dry mass at 0.0003, 0.0006, 0.0009, 0.0012, 0.0015 and 0.0018 g/ml. Yeast fermented samples were measured the absorbance of the samples with the spectrophotometer at 600 nm (Bausch and Lomb spectrophotometer, VWR Scientific Inc. N.Y.) and then used regression equation for determined yeast number (cell/ml) from absorbance.

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, ether extract (EE) and crude protein (CP) determined according to AOAC (1995). The samples were also analyzed for neutral-detergent fiber (NDF) and acid-detergent fiber (ADF) according to Van Soest et al. (1994). YBM4 was analyzed for amino acid profile at Central Laboratory (Thailand) Co., Ltd (In house method based on AOAC Official Method 994.12, 988.15v (2000).

Statistical analysis

Kinatics of yeast growth and chemical composition of YEFECAP were statistically analyzed using analysis of variance of a Completely randomized design with a 2x4 factorial arrangement using Proc. GLM procedure (SAS, 1998). Treatment means were statistically compared using Duncan's New Multiple Range Test (Steel and Torrie, 1980).

Results

Standard curves

The standard curves were produced from 8 treatment combinations at 0 h. Figure 1. shows the relationship between absorbance values and number of cells. It was found that when increasing concentration of yeast, absorbance value and the number of cells were increased. The linear relationship between cell numbers and absorbance values are illustrated in Figure 1. The values of r^2 of YBM1,YBM2,YBM3,YBM4,YCM1,YCM2,YCM 3 and YCM4 were 0.92, 0.97, 0.96, 0.98, 0.92, 0.98, 0.98 and 0.94, respectively. This result implied that regression equation could use to determine yeast number (cell/ml) from absorbances.

Table 1. Kinetic of growth of the different type of yeasts and different mediums in this experiment (x 10^{11} cell/ml).

Cultivation	YB				YC					Contrast[1]		
Time (h)	M1	M2	M3	M4	M1	M2	M3	M4	SEM	A	B	A*B
0	0.5	0.4	0.5	0.5	0.4	0.4	0.5	0.4	0.07	ns	ns	ns
6	0.5	0.5	0.6	0.6	0.5	0.4	0.5	0.4	0.07	ns	ns	ns
12	0.6	0.6	0.8	0.6	0.6	0.5	0.6	0.6	0.09	ns	ns	ns
18	0.8^{ab}	0.8^{ab}	0.9^{ab}	0.9^{a}	0.7^{ab}	0.6^{b}	0.6^{ab}	0.6^{b}	0.09	**	ns	ns
24	1.0^{a}	1.1^{a}	1.1^{a}	1.0^{ab}	0.7^{b}	0.7^{b}	0.7^{b}	0.7^{b}	0.10	**	ns	ns
30	1.1^{ab}	1.2^{a}	1.2^{a}	1.1^{a}	0.9^{ab}	0.8^{b}	1.1^{ab}	1.1^{ab}	0.08	*	ns	ns
36	1.4	1.6	1.4	1.3	1.3	1.2	1.2	1.1	0.13	ns	ns	ns
42	1.4^{c}	1.9^{a}	1.8^{ab}	1.5^{bc}	1.6^{bc}	1.6^{abc}	1.7^{abc}	1.4^{c}	0.10	ns	*	ns
48	1.9	2.2	2.3	1.9	1.8	2.0	2.0	1.9	0.12	ns	ns	ns
54	2.2^{bc}	2.6^{ab}	2.7^{a}	2.2^{bc}	2.0^{c}	2.4^{abc}	2.4^{abc}	2.0^{c}	0.13	ns	*	ns
60	2.2^{de}	2.8^{ab}	3.0^{a}	2.3^{cde}	2.1^{e}	2.5^{bcd}	2.7^{abc}	2.2^{de}	0.12	ns	**	ns
66	2.1^{c}	2.7^{ab}	3.1^{a}	2.2^{c}	2.1^{c}	2.5^{bc}	2.8^{ab}	2.2^{c}	0.14	ns	**	ns
72	2.1^{bc}	2.7^{abc}	3.0^{a}	2.2^{bc}	2.1^{c}	2.5^{abc}	2.8^{ab}	2.2^{bc}	0.19	ns	**	ns
78	2.2^{c}	2.7^{ab}	3.1^{a}	2.2^{c}	2.1^{c}	2.5^{bc}	2.8^{ab}	2.2^{c}	0.13	ns	**	ns
84	2.1^{c}	2.7^{ab}	3.0^{a}	2.2^{c}	2.1^{c}	2.5^{bc}	2.8^{ab}	2.2^{c}	0.14	ns	**	ns
90	2.1^{d}	2.7^{b}	3.0^{a}	2.2^{cd}	2.1^{d}	2.5^{bc}	2.8^{b}	2.2^{d}	0.08	*	**	ns
96	2.1^{c}	2.7^{ab}	3.0^{a}	2.2^{c}	2.1^{c}	2.5^{bc}	2.7^{ab}	2.2^{c}	0.13	ns	**	ns
102	2.1^{d}	2.7^{bc}	3.0^{a}	2.2^{d}	2.1^{d}	2.5^{c}	2.7^{b}	2.2^{d}	0.07	*	**	ns
108	2.1^{e}	2.7^{bc}	3.0^{a}	2.2^{de}	2.0^{e}	2.5^{cd}	2.7^{b}	2.2^{e}	0.08	*	**	ns
114	2.1^{d}	2.7^{b}	3.0^{a}	2.2^{d}	2.0^{d}	2.4^{c}	2.7^{b}	2.1^{d}	0.06	**	**	ns
120	2.1^{cd}	2.7^{ab}	3.0^{a}	2.2^{cd}	2.0^{d}	2.4^{bc}	2.7^{abc}	2.2^{cd}	0.11	ns	**	ns

abcde 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<0.05, **P<0.01. (Calculated from regression equation).

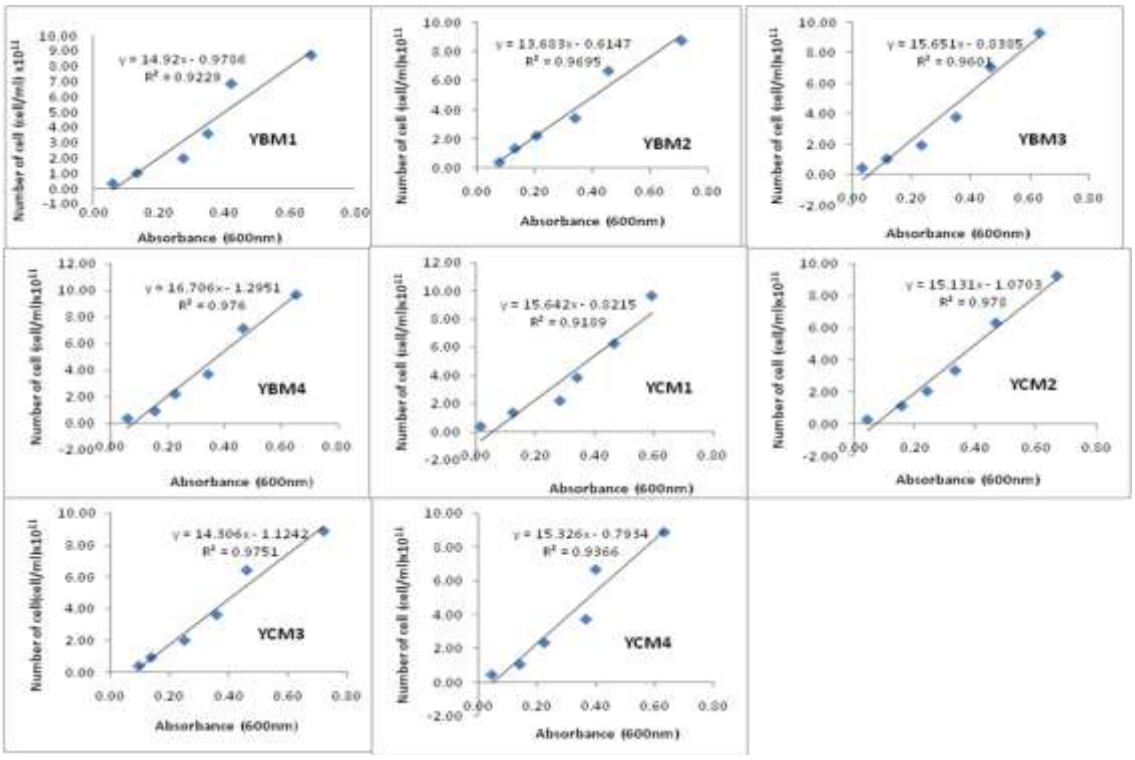

Figure 1. Standard curve and regression equation of each treatment combinations.

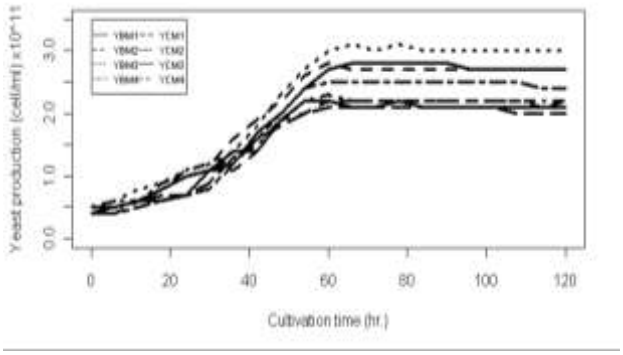

Figure 2. Effects of different yeast type and mediums on kinetics of yeast growth.

Effects of different yeast type and different medium on kinetic of yeast growth

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 at 27°C (Table 1 and Figure 2). 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 the highest ($P<0.05$) in number at $3x10^{11}$cell/ml while YBM1 and YCM1 were the lowest ($P<0.05$) in number $2.1x10^{11}$cell/ml.

Effect of medium on kinetic of yeast growth

Figure 3 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 showed significant differences ($P<0.05$) at 42, 54, 60, 66, 72, 78, 84, 90, 96, 102, 108, 114 and 120 h post-cultivation by yeast growth of medium M1, M2 and M3 were increased ($P<0.05$) when increasing proportion of urea, and the highest ($P<0.05$) in medium M3, while in medium M4 resulted in a poor result.

Effect of yeast type on kinetic of yeast growth

Effect of yeast type on kinetic of yeast growth is shown in Figure 4. It was found that growth of Baker's yeast (YB) was significantly ($P<0.05$) higher than Brewer's yeast (YC), especially at 18, 24, 30, 90, 102, 108 and 114 h post-cultivation.

Effects of different yeast type and medium on chemical composition of YEFECAP products

Effects of different yeast type and medium on chemical composition of yeast fermented cassava chip protein (YEFECAP) product are shown in Table 2. It was found that interaction between yeast type and medium was significantly ($p<0.01$) influenced on percentage of DM, OM, CP and ADF. DM of YBM1 had the highest DM (92.1%) and YCM1 and YCM2 were the lowest (90.1%), while OM of YBM3 was the highest (97.3%) and YCM4 was the lowest (96.0%). The mean protein value of YEFECAP products; YBM4 was the highest followed by YBM3, YCM4, YBM2, YCM3, YCM2, YBM1 and YCM1 and the 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 ($P>0.05$) in EE and NDF contents of the YEFECAP products. Amino acid profile of YBM4 is shown in Figure 5. It was shown that lysine was the highest followed by leucine, glutamic acid, isoleucine, valine, tyrosine, alanine, aspartic acid, histidine, glycine, proline, threonine, methionine and tryptophan respectively, while arginine, cystine and hydroxylysine were the lowest.

Discussion

Standard curves

Percentage of variation in response of cell/ml to factors of absorbance value measured by spectrophotometer and the cell numbers of direct cell count were closely related accordingly. Therefore, the regression equations between spectrophotometer and direct counts from standard curves could be used in this experiment.

Table 2. 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	**	**	**

abcdefgh 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, **$P<0.01$.

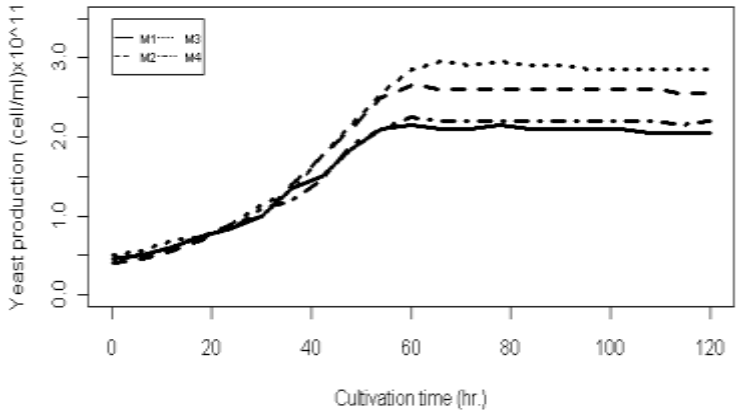

Figure 3. Effects of mediums on kinetics of yeast growth.

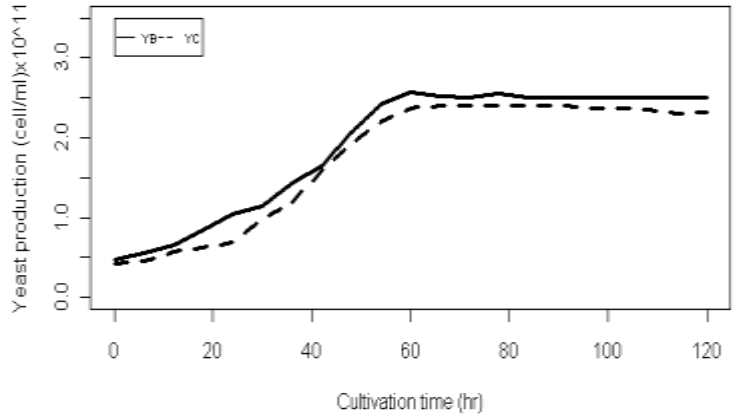

Figure 4. Effects of yeast type on kinetics of yeast growth.

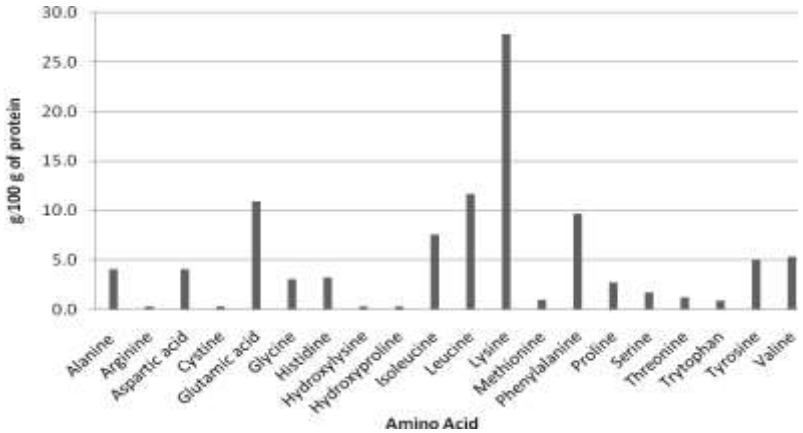

Figure 5. Amino acid profile of YEFECAP products in YBM4 (Baker's yeast with urea: molasses: water ratio at 64:08:100 (M4)).

Effects of different yeast type and medium on kinetic of yeast growth

Concerning kinetic of growth of Baker's yeast and Brewer's yeast, it was shown that in the first 0-6 h from the beginning, the growth was slow because the cells were adapted to the new surrounding medium. The volume of cells was swelling and the metabolism of cell was increased, but the proliferation of cell was slow, as a lag phase. After lag phase, the growth became rapidly increased because cells can adapt to the surrounding medium, and the substances can pass

through cell than in the lag phase. Hence, the growth of yeast was increased by budding cells in this phase, as an exponential phase. The budding cell cycle is the succession of events, whereby a cell grows and divides into two daughter cells that each contains the information and then, repeats the process. The pH and temperature in this study were similar with the 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 (Wang et al., 2004; Pramanik and Roa, 2005; Asli, 2010; Manikandan and Viruthagri, 2010).

In this study most of the treatments resulted in the 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 in number $2.1x10^{11}$cell/ml, that could be an effect of the from different medium ($p < 0.05$). At the same time of cultivation yeast and interaction between yeast type and medium had no affect ($P>0.05$) on yeast numbers. This result agreed with the work of Wang et al. (2004), Pramanik and Roa (2005) and Asli (2010) who reported that optimal cultivation time of *S. cerevisiae* was approximately 60 to 80 h by highest biomass.

Effect of mediums on kinetic of yeast growth

The growth curve of yeast *S. cerevisiae* (Baker's yeast and Brewer's yeast) in medium M1, M2 and M3 with increasing proportion of urea, yeast growth were higher and the highest in medium M3, while medium M4 resulted in poor result. Manikandan and Viruthagiri (2010) reported that optimum carbon and nitrogen ratio (C:N) of cultivation medium of *S. cerevisiae* was found to be 35.2 which yielded a maximum ethanol and cell mass. Whereas, Danesi et al. (2006) used yeast extract as nitrogen source and molasses as carbon source, it was found that optimal C:N ratio for recombinant *S. cerevisiae* cultivation was 10.

In this study, the poor growth in medium M4 could be explained that the concentration of urea was not optimum, and large amount of urea might exert a toxic effect. An appropriate amount of C:N ratio was the key to harvest maximum microbial biomass protein (Zheng et al., 2005). However, generally the results confirmed that urea, a low cost fertilizercould supported maximum microbial biomass protein production which confirmed from previous findings (Ali et al., 2009). Furthermore, utilization of sucroses or glucose as carbon source is not economical in the production of microbial biomass protein and a less expensive carbohydrate source would be beneficial. Low cost substrates such as cane molasses can be used for the production of microbial biomass protein for animal feed supplements (Sattar et al., 2008). Molasses, a cheap by-product, is widely available from the sugar industry and consist of water, sucrose (47-50%, w/w) which is the disaccharide most easily utilized by yeast cells, 0.5-1% of nitrogen source, proteins, vitamins, amino acids, organic acids and heavy metals (Roukas, 1998). Hence, it is a very attractive carbon source for microbial biomass protein production by mixed culture from economic point of view.

Effect of yeast type on kinetics of yeast growth

The growth of Baker's yeast (YB) was higher ($P<0.05$) than Brewer's yeast (YC). That could be due to Brewer's yeast is by product from beverage industry which might contain some of waste product from yeast metabolism which could inhibit yeast growth. However, potential of *S. cerevisiae* (Baker's yeast and Brewer's yeast) has been widely used for protein production (Lang et al., 1997). For the two yeast, they showed fast growth, short generation time, a border range of materials may be considered as suitable substrates depending on the microorganism chosen especially, can be grown on media containing cheap sources of C and N for efficient yield (Reed and Nagodawithana, 1995).

Effects of different yeast type and medium on chemical composition of YEFECAP products

The increase in growth and proliferation of fungi or bacterial complex in the form of single cell proteins may possibly account for the apparent increase in the protein content and also found by Oboh (2006). Moreover, Aro (2008) reported that microbial fermentation with cassava starch residue could improve nutritive quality by increasing CP, EE and ash and decreased cyanide, CF, NDF and ADF. This high protein content could be attributed to the ability of the *S. cerevisiae* to secret some extracellular enzymes such as amylases, linamarase and cellulase in to cassava mash during their metabolic activities, which would lead to yeast growth (Oboh and Akindahunsi, 2003). The protein contents of product (Table 2) 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 also yeast fermented liquid and cassava ratio was higher than those reported by Boonnop et al. (2009) and Wanapat et al. (2011). YBM4 contained essential amino acid especially lysine, this result is similar with Yamada and Sgarbieri (2005) reported that whole cell of *S. cerevisiae* was high in amino acid profile especially

lysine. Even though, kinetic of yeast growth in YBM3 in this study was the highest ($P<0.05$) but when considering the crude protein content of YEFECAP products, it was lower than in YBM4. It could be due to NPN level in YBM4 that was higher than in YBM3, which is a good N source use for synchronized soluble carbohydrates in the rumen of ruminants (NRC, 2001; Wanapat, 2003; Wanapat and Khampa, 2007). Moreover, many researchers studied about synchronizing urea and cassava chip by processing a product such as cassadro (Poungchompu, 2000) and U-cal (Cherdthong, 2010, 2011).

Conclusions and Recommendations

Based on the results of this experiment, it could be concluded that growth kinetic of *Sacchromyces cerevisiae* in YBM3 (Baker's yeast with urea:molasses:water ratio at 56:16:100 (M3)) was the 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 the highest in protein content at 47.5% CP with high in lysine. However, further research is required to study the use of YEFECAP as a protein source to replace soybean meal in *in vivo* feeding trials in productive ruminants.

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