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The amino acid profile of yeasts from ketchup factory waste as a candidate of single cell protein (SCP)

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Abstract

Yeast can be used as a feed additive to increase livestock health in the form of probiotic and immunostimulant. This research aims to observe the protein content and amino acid profile of yeast isolated from ketchup factory waste in East Java, Indonesia. The yeast biomass production was carried out in yeast malt broth medium containing 3% glucose, 0.3% malt extract, 0.5% bacto peptone and 0.3% yeast extract. Protein content in each yeast isolate was measured using Bradford method and the amino acid profile was estimated using High Performance Liquid Chromatography (HPLC). From seven yeast isolates detection resulted the highest protein content was KYP3K2 isolate containing 1172.33 µg/ml of protein. Besides, both isolates KYP3K2 and AYP6K2 have low nucleic acid content of 15.8 %. Amino acid profile showed that both samples consist of nine essentials amino acid and seven non essentials amino acid. In conclution, both samples fully recommended as Single Cell Protein as feed livestock.

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Introduction

Increasing of human population caused increase of meat demand for consumption. Animal is one of protein source that high consume by people. In Indonesia, most people consume poultry meat or egg as a protein main source. So that implicate to poultry meat demand from 1.4 billion in the 2010 and will be increase up to 1.5 billion in the 2011 (Directorate General of Livestock and Animal Health Ministry of Agriculture Indonesia, 2012). Animal feed is the most factors that effect to increasing the quantity and quality of the poultry meat.

Protein has high nutritional value for the animal feed. Protein deficiency in the animal feed can be caused obstruct animal growth. Feed quality can be determined from its content such as protein, lipid, fiber, carbohydrate, vitamins, minerals, and water content. Fish powder and soy slag has high protein content and complete amino acid. Nonetheless, animal feed still has the high price. It is because of import of the raw material. So that, we must find the other resource for the animal feed.

Ketchup factory waste consists of liquid and solid waste materials. The solid waste consists of protein, fat and fiber. Therefore, it is very useful to utilize it to explore microorganism in that waste. The previous research related with isolation of yeast from solid waste of ketchup factory and their potency to produce ethanol had been done (Putri and Ardyati, 2013). However, research about the amino acid content from those yeasts has not yet analyzed. In this research we focused on the profile of amino acid content from yeast isolates which is used as candidate of SCP.

In the 1996, the single cell protein (SCP) was defined as protein source especially form yeast, bacteria, and algae. The SCP known as an alternative protein source for food and feed (Nasseri *et al*., 2011; Khan *et al*., 1992). From the other candidate, yeast is the most competent as SCP because of high bioactive compound such as protein, amino acid, vitamins, polysaccharide, lipid acid, phospholipids, polyamine, astaxanthines, β-carotene, trehalose, glutathione, superoxide dismutase, chitinase, amylase, phytase and lipid (Yoseph, 2009).

Yeast can use waste product as carbon source for its growth. Waste product can change into biomass, protein concentrate or amino acid by specific protease (Bhalla *et al*., 2007). The waste product management for SCP carbon source has a good prospect for business. Thus this research was conducted to know the protein content and amino acid profile from yeast biomass from solid ketchup waste.

Material and methods

Sample collection

Samples were collected from three location, they are PT Indofood factory namely I2YP5K1 and I2YP5K2; home industry ketchup from Kediri were namely KYP6K1 and KYP3K2; and the last PT ABC Pandaan were namely AYP6K1, AYP6K2, dan AYP5K4.

Screening of isolates for biomass production

One oose of each isolate was inoculated into Yeast Malt Broth (YMB) medium and incubated at shaker rotary for 48 h at 30 ^{0} C. Yeast starter was taken 10 ml and input to 90 ml YMB medium and incubated in the shaker rotary for 120 rpm, at 30 ^{0} C about 48 h. Yeast biomass was collected by centrifuged the inoculants for 5000 rpm, 4°C, 5 min. Supernatant was discarded and pellet was washed there times using PBS.

The yeast culture in the exponential growth phase was pelleted by centrifugation at 5,000 rpm for 5 minutes at 4°C,. The pellet was washed with phosphate buffered saline (PBS) to eliminate medium contamination. Crude protein extracts of the yeast were prepared by rapidly homogenizing approximately 1 g of the yeast pellet (washed with phosphate buffered saline) using a mortar and pestle in liquid nitrogen and 2 ml protein extraction buffer containing 50 mM Tris-HCl, pH 7.4, 1 mM EDTA, 2 mM MgCl2 , 2 mM DTT, 2.5 mM PMSF, 0.1 per cent Triton-X-100. The homogenate was centrifuged at 12,000 rpm for 10 minutes at 4°C. The supernatant

was collected and protein was estimated (Bhima *et al.*, 2011).

Protein Estimation

Protein concentration of yeast protein extracts was determined using the Bradford protein assay according to the manufacturer's instructions and using BSA as a standard. BSA standard was made by diluting the stock of 10mg/ml to a final concentration of 1mg/ml. Clean, autoclaved glass tubes were taken, labeled and added 0,1 ,2, 3, 4, 5, 6, 7, 8, 9, 10 µg BSA respectively. The volume in each tube was made to 800 µl with double distilled water. Two hundred µl of Bradford reagent was added to these tubes to make up to a final volume of 1ml and vortexed for few seconds. The same process was repeated for unknown samples. Each unknown was taken in duplicates or triplicates. The tubes were incubated at room temperature for 10 minutes (in dark) and the samples were read at 595 nm using spectrophotometer against a reagent blank. A standard curve was plotted and protein concentration of the unknown samples was estimated from the standard curve (Bhima *et al.*, 2011). The protein content was estimated based on formula1.

$$K = \frac{BEP}{PS} x \frac{KP}{BP} \qquad (1)$$

K : protein concentration (µg/g)
BEP : buffer extract volume (µL)
PS : Supernatant protein volume (µL)
KP : protein concentration
BP : pellet weight (g)

Nucleic Acid Estimation

Nucleic acid was estimated based on spectrophotometric technique using UV absorbance at 260 and 280 nm. About 10 µL protein supernatant was added into 990 µL PBS buffer and then homogenize by pippetting. The mix solution was absorbed at 260 and 280 nm. The nucleic acid was estimated by counted ratio of A280 and A260 and compare with nucleic acid ration based on Robyt and White (1987) tables.

Amino acid characterization

The amino acid was determined using High Performance Liquid Chromatography (HPLC) in the laboratory Central of life science, Brawijaya University Indonesia based on Waters method.

Biochemistry test using API 20 C AUX kit

The yeast was inoculated in the Yeast Malt Agar medium using quadrant streak method. The yeast was incubated at 30 °C for 48 h. the single colony was formed was taken using cotton swab and inoculated into 3 mL NaCl 0.85 %. The turbidity level was compared by McFarland standard buffer. The yeast suspension about 100 µl was inoculated into API C medium and homogenized using sterile pipette. The inoculants were transferred into cupule at API C AUX strip. The inoculants were incubated at 30 °C for 48 - 72 h (Lee *et al.*, 2011).

Results and discussion

Biomass protein content of yeast isolates

Yeast biomass production is done with three repetitions. The protein content is measured per g of yeast isolates pellets. The average protein content of all yeast isolates is presented in Figure 1.

The data showed that the yeast isolates I2YP5K1, I2YP5K2, and AYP5K4 has a high standard deviation values. It is also evident from the descriptive analysis of box plots (Figure 2). Descriptive analysis using box plots show that the variation of the protein content of yeast isolates I2YP5K1, I2YP5K2, and AYP5K4 too high. Thereby to determine the yeast isolates that have a high protein value of different test for yeast isolates KYP6K1, KYP3K2, AYP6K1 and AYP6K2.

Different test results with one-way ANOVA followed by Tukey's test (HSD) showed that the yeast isolates KYP3K2, and AYP6K1 have the highest levels of real protein isolates KYP6K1 then have the lowest levels whereas real protein isolate has a protein value AYP6K2 them so as to further test analysis amino acids selected isolates KYP3K2 and AYP6K2 (Figure 3). KYP3K2 isolates representing the highest protein

content while AYP6K2 isolates representing low and high protein content.

Nucleic acid content in the SCP depends on the type of microorganisms that produce them. Nucleic acid content criteria used for SCP production ranged 1-11%. High nucleic acid content to be a problem in the use of SCP. Degradation of nucleic acids in poultry produce uric acid. High content of uric acid causes disorders of the kidneys due to poultry has a low content of uricase enzyme (Nasseri *et al*., 2011).

Tabel 1. Amino acid content of yeast isolates KYP3K2 and AYP6K2.

Types of amino acid	Yeasts isolates			
	KYP3K2		AYP6K2	
	Essential amino acids	Non-essential amino acid	Essential amino acids	Non-essential amino acid
L-Histidine	0,275		0,306	
L-Arginin	0,596		0,661	
L-Threonine	0,530		0,543	
L-Valine	0,708		0,667	
L-Metheonine	0,149		0,173	
L-Lysine	0,953		0,924	
L-Isoleucine	0,544		0,554	
L-Leucine	0,731		0,753	
L-Phenylalanine	0,502		0,545	
L-Aspartic acid		0,775		0,878
L-Serin		0,495		0,522
L-Glutamic acid		1,313		1,311
L-Glycine		0,561		0,535
L-Alanin		0,577		0,697
L-Prolin		0,527		0,795
L-Tyrosine		0,346		0,392
Total	4,988	4,594	5,126	5,13
% total amino acid	52,1	47,9	50,0	50,0

Seventh nucleic acid content of yeast isolates (Figure 4) were categorized as high as 15.80% range - 19% nucleic acid content is in accordance with the nucleic acid content of yeast described by Roth which ranges from 13% - 20% (Roth, 1980). Based nukleatnya acids, yeast isolates KYP3K2 and AYP6K2 have nucleic acid content lower than other yeast isolates, the two isolates were selected for amino acid analysis.

Amino acid profiles of yeast isolates

Types of amino acids contained in the yeast isolates and AYP6K2 KYP3K2 determined by comparing the retention time of standard amino acids with retention time of the samples tested. Retention time is the time required by the sample from the time of injection until the sample reaches a maximum peak (Riyadi, 2009). Peak amino acids that are tested will have the same retention time values with standard retention time values.

KYP3K2 yeast isolates and AYP6K2 produce 16 types of amino acids. Amino acids consist of nine essential amino acids and seven non-essential amino acids (Figure 5 and table 1). Essential amino acids contained in KYP3K2 and AYP6K2 is histidine, arginine, threonine, valine, methionine, isoleucine,

leucine, phenylalanine, lysine. Non-essential amino acid found in yeast isolates and AYP6K2 KYP3K2 is aspartic acid, glutamic acid, serine, glycine, alanine, proline and tyrosine.

The content of essential amino acids were highest in yeast isolates and AYP6K2 KYP3K2 is the value of 0.953% lysine and 0.924%. While the essential amino acid content of the lowest in the two isolates was methionine with a value of 0.149% and 0.173%. Essential amino acids are amino acids that cannot be synthesized by the body or synthesized too slowly to meet the metabolic requirements, and established as an important dietary element.

Table 2. Biochemical characters and AYP6K2 KYP3K2 isolates with the API 20 C AUX.

Subtrate	*Saccharomyces cerevisiae strain 1**	*Saccharomyces cerevisiae strain 2**	Isolates	
			KYP_3K_2	AYP_6K_2
GLU	+	+	+	+
GLY	-	-	-	-
2KG	-	-	-	-
ARA	-	-	-	+
XYL	-	-	-	+
ADO	-	-	-	+
XLT	-	-	-	-
GAL	+	+	+	+
INO	-	-	-	-
SOR	-	-	-	-
MDG	-	-	+	+
NAG	-	-	-	+
CEL	-	-	-	+
LAC	-	-	-	+
MAL	+	+	-	+
SAC	+	+	+	-
TRE	-	+	-	+
MLZ	-	+	-	-
RAF	+	+	+	-
HYPH	-	-	-	-
% ID			99,7	Unidentified

GLU= glucose; GLY= glycerol; 2KG= 2-keto-D-gluconate; ARA= arabinose; XYL= xylose; ADO= adonitol; XLT= xylitol; GAL= galactose; INO= inositol; SOR= sorbitol; MDG= methyl-D-glucoside; NAG= N-acetyl- glucosamine; CEL= cellobiose; LAC= lactose; MAL= maltose; SAC= saccharose; TRE= trehalose; MLZ= melezitose; RAF= rafinose; HYPH= hifa

* Data from package insert API 20 C AUX as standard

Essential amino acid for poultry is lysine, methionine, threonine, tryptophan, isoleucine, leucine, histidine, valine, phenylalanine, and arginine. Non-essential amino acids are amino acids that can be synthesized by the body so it does not need to be considered in feed formulation. Cysteine and tyrosine are considered a semi-essential amino acid; because cysteine can be synthesized from methionine and tyrosine are synthesized from phenylalanine. Amino acids lysine and methionine is the limiting amino acid in most poultry diets. These amino acids must be supplied in food or feed (Ravindran, 2013).

Lysine requirement ranges from 0.45 % - 0.85 % methionine and 0.10 to 0.32% (Parkhurst and Mountney. 1988). The content of lysine of the two yeast isolates analyzed were from 0.924 to 0.953 % yeast showed that both isolates are best used as a source of lysine for poultry feed. Content of methionine from both yeast isolates analyzed were 0.149 to 0.173 % yeast isolates showed that both can be used as a source of methionine for poultry feed. Amino acid requirements of poultry is influenced by several factors, including production levels, genotype, sex, physiological, environmental and health for example, to increase the deposition of high meat

requires relatively high in lysine, while egg production or to increase hair growth requires relatively high methionine.

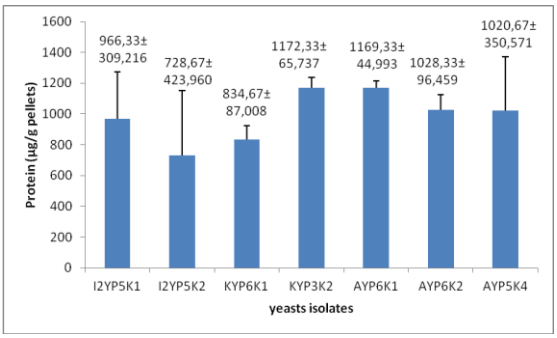

Fig. 1. The average protein content of all yeast isolates per gram pellets.

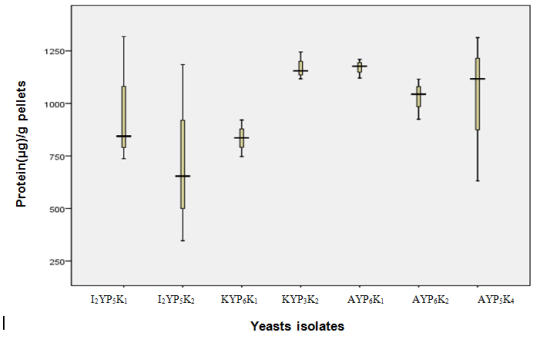

Fig. 2. Variation in protein content of yeast isolates based on analysis of box plot.

Both of these isolates have the essential amino acids that are needed in growing broilers and laying hens. Based on the needs of protein and amino acids in broilers and age of laying hens (NRC, 1994) then the yeast isolates AYP6K2 KYP3K2 and can meet the needs of the amino acids lysine and histidine for broilers and laying hens. Glycine and serine requirement for broilers 6-8 weeks of age can be met by both yeast isolates. Phenylalanine, phenylalanine + tyrosine and threonine produced by both isolates was also meets the needs of laying hens. KYP3K2 yeast isolates can meet the needs of the amino acid valine for broilers aged 6-8 weeks and laying hens.

Biochemical tests of yeast isolates

Biochemical test results and AYP6K2 KYP3K2 isolates and analyzes using databases apiweb™ software is presented in Table 2. The results showed that KYP3K2 a strain of *Saccharomyces cerevisae* 1 with ID 99.7%, while the isolates AYP6K2 unidentified.

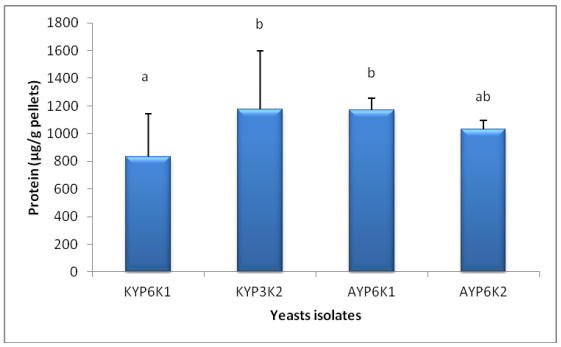

Fig. 3. The average protein content of four yeast isolates Description: The same notation indicates not significantly different by Tukey test (HSD) at α 0.05.

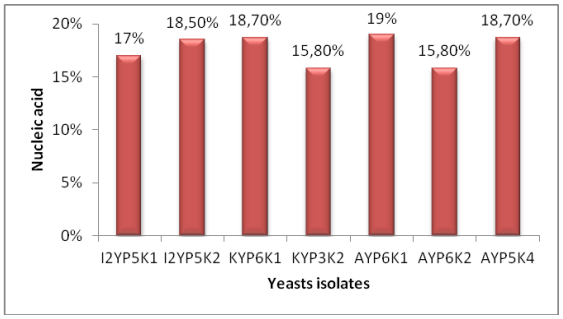

Fig. 4. Average nucleic acid content of yeast isolates.

Based on the characteristics of the amino acids that are owned by yeast isolates KYP3K2 and AYP6K2, then both these isolates can be used as a candidate in the form of feed additive SCP. Isolate yeast *Saccharomyces cerevisae* KYP3K2 is, it isolates can be used as a feed additive to improve feed quality and also as an immunostimulant.

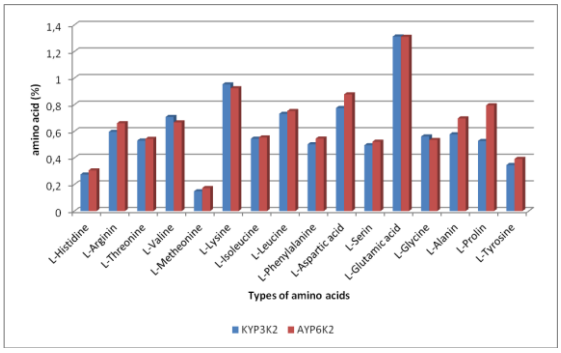

Fig. 5. Amino acid content of yeast isolates KYP3K2 and AYP6K2.

The addition of live yeast culture (*Saccharomyces cerevisiae*) to the contaminated feed aflaktosin can protect broilers from aflatoxicosis harmful effects. Provision of yeast culture residue (YCR) on chicken feed boiler aflaktosin can neutralize harmful effects

(Stanley *et al*., 2004). YCR is made from the cell wall of *Saccharomyces cerevisiae*. *Saccharomyces cerevisiae* cell wall glucan and some molecules containing glucose. Glucans present in the inner cell wall consists of a natural complex carbohydrates that enhance the immune response by activating phagocytic cells, trap and engulf foreign cells (immunostimulatory).

Conclusion

KYP3K2 yeast isolates, and AYP6K1 have the highest levels of real protein isolates KYP6K1 then have the lowest levels of real protein while AYP6K2 have value protein isolates them. Amino acid profiles of yeast isolates KYP3K2 and AYP6K2 has nine essential amino acids and seven non-essential amino acids with different levels. Both isolates potentially as single cell protein candidates (SCP) to improve the quality of animal feed in the form of a feed additive.

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