



**Addition of Carbon Sources to Pineapple Waste Media in the Production of Single Cell Protein Biomass *Saccharomyces cerevisiae***

*Penambahan Sumber Karbon Pada Media Limbah Nanas dalam Pembuatan Protein Sel Tunggal Biomassa *Saccharomyces cerevisiae**

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**Abstract**

Single-cell protein (SCP) is the term used for crude or pure protein derived from simple single or multi-celled microorganisms. Pineapple peel contains monosaccharides as much as 10.8% so that it can be used as a fermentation medium in single-cell protein production. The purpose of this study was to determine the effect of adding carbon sources of fructose and sucrose on pH, cell dry weight, and protein content in the manufacture of single-cell proteins. This study used a completely randomized design (CRD) with two factors, namely the addition of carbon (fructose, sucrose, and control) and fermentation time (24.48, and 72 hours). The data analysis used the variance test and the Duncan Multiple Range Test (DMRT) continued to test with a confidence level of 95%. The results showed that the addition of carbon to the media had a very significant effect on media pH, cell dry weight, and protein content. In the medium with the addition of fructose it has a pH of 3.81; dry weight 0.4203 grams; and protein content 69.08/L. Whereas in the media with the addition of sucrose, the pH was 4.33, the dry weight of the cells was 0.3385 grams, and the cells had a protein content of 85.55 mg/L. The addition of a fructose carbon source gave the cell dry weight more than the addition of carbon sucrose

**Abstrak**

*Protein sel tunggal (PST) adalah istilah yang digunakan untuk protein kasar atau murni yang berasal dari mikroorganisme bersel satu atau banyak yang sederhana. Kulit buah nanas mengandung monosakarida sebanyak 10,8% sehingga dapat dimanfaatkan sebagai media fermentasi pada produksi protein sel tunggal. Tujuan penelitian ini adalah untuk menguji pengaruh penambahan sumber karbon fruktosa dan sukrosa terhadap pH, berat kering sel, dan kadar protein dalam pembuatan protein sel tunggal. Penelitian ini menggunakan Rancangan Acak Lengkap (RAL) dua faktor yaitu penambahan karbon (fruktosa, sukrosa, dan kontrol) dan waktu fermentasi (24,48, dan 72 jam). Analisis data menggunakan uji sidik ragam dan uji lanjut Duncan Multiple Range Test (DMRT) dengan taraf kepercayaan 95%. Hasil penelitian menunjukkan bahwa penambahan karbon pada media memberikan pengaruh yang sangat nyata terhadap pH media, berat kering sel dan kadar protein. Media dengan penambahan fruktosa memiliki pH sebesar 3,81; berat kering 0,4203 gram; dan kadar protein 69,08/L. Pada media dengan penambahan sukrosa menghasilkan pH sebesar 4,33 dengan berat kering sel sebanyak 0,3385 gram, dan sel memiliki kadar protein sebesar 85,55mg/L. Penambahan sumber karbon fruktosa memberikan berat kering sel lebih banyak dibandingkan dengan penambahan karbon sukrosa.*

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## INTRODUCTION

The increasing population of humans and animals causes the demand for food and animal feed to increase. Food and animal feed must contain good nutritional elements such as protein. A large number of protein needs for humans and animals results in proteins derived from animals and plants that are not sufficient for human and animal populations. Since the 1950s, efforts have been made to find alternative sources of protein. Among them are single-cell proteins derived from microorganisms. Proteins obtained from microorganisms are cheap and compete with other protein sources and can provide good nutritional value depending on the amino acid composition (Dhanasekaran et al., 2011).

Single-cell protein is a term used for crude or pure protein derived from unicellular or multicellular microorganisms, such as bacteria, yeast (yeast), fungi, algae, and protozoa. The microorganism used in this study was *S.cerevisiae* yeast. When compared to the production of protein from animals and plants, single-cell protein production is more efficient, because it does not require a large area, does not cause waste, and the production process is fast, reproduction of microorganisms such as bacteria and yeast can give greater yields every hour, while algae take less than one day (Pawignya, 2011).

Single-cell proteins can be produced on several different substrates. Conventional substrates such as starch, molasses, fruit, and vegetable waste have been used for the manufacture of single-cell proteins (Suman et al., 2015; Srividya et al., 2014). Monosaccharide and disaccharide substrates are mostly used for the manufacture of single-cell proteins because monosaccharides and disaccharides are natural microbial substrates and are renewable raw materials.

Pineapple fruit is widely used by most Indonesians for consumption needs. Apart from being consumed fresh, pineapples are also widely used as raw material for the agricultural industry with various products such as processed pineapples, such as jam, sweets, syrup, lunkhead, chips, canned fruit, which are Indonesia's leading export products (Nastiti et al., 2013). From the various types of processing, pineapple waste will be obtained in large enough quantities. Pineapple waste consists of peel waste and pineapple stem waste. Waste or by products of pineapple are still limited in use and relatively just thrown away (Tahir et al., 2008).

The nutritional content of pineapple peels includes water 84.50%, reducing sugar 6.62%, protein 6.4%, and crude fiber 16.7% (Nastiti et al., 2013). The sugar content in pineapple peel can be used in making single-cell protein. Because microorganisms will use this sugar as a carbon source and as an energy source. Carbon sources that can be added to the manufacture of single-cell proteins are monosaccharides and disaccharides (Pawignya, 2011). Meanwhile, Purwitasari et al. (2004) in her study stated that the carbon source added to the manufacture of single-cell proteins comes from coconut water which contains glucose, sucrose, fructose, inositol, and sorbitol. Then, the research of Pawignya (2011) and Masithoh (2012) used sucrose as a carbon source in a single cell protein maker. Therefore this study aims to determine the effect of adding types of carbon sources on pH, cell dry weight, and protein content in the manufacture of single-cell proteins. The carbon sources added in this study were fructose and sucrose. Fructose is one of the monosaccharide carbohydrates, while sucrose is one of the disaccharide carbohydrates.

## MATERIALS AND METHODS

This research was conducted at the Quality Control Laboratory, Agricultural Product Processing Technology Laboratory and Instrument Laboratory Department of Teknologi Agroindustri, FPTK Universitas Pendidikan Indonesia Bandung.

This research was conducted using two-factor completely randomized design (CRD) experiments, namely the type of carbon source and fermentation time. The carbon source factor consists of three treatments, namely no addition of carbon, the addition of fructose, and the addition of sucrose. While, the fermentation time factor consisted of three fermentation time treatments, namely 24 hours, 48 hours, and 72 hours.

The tools used in the manufacture of this single-cell protein are autoclave, shaking incubator, 250 ml Erlenmeyer, spirit burner, laminar air flow cabinet, screw tube, loop needle, micropipette. Meanwhile, the tools used for single-cell protein analysis are pH meter, centrifuge, analytical balance, oven, desiccator, UV-VIS spectrophotometer, and cuvette.

The materials used in the manufacture of this single-cell protein are pineapple peel waste, as a source of *S. cerevisiae*, sucrose, fructose, PDA media, aquadest,  $(\text{NH}_2)_2\text{SO}_4$ ,  $\text{KH}_2\text{PO}_4$ ,  $\text{MgSO}_4$ ,  $\text{CaCl}_2$ ,  $\text{CH}_3\text{COOH}$ ,  $(\text{CuSO}_4 \cdot 5\text{H}_2\text{O})$  and  $\text{KNaC}_4\text{H}_4\text{O}_6$ . While,

the materials used for the single-cell protein analysis test are *Bovine Serum Albumin* (BSA) Standard Solution (1; 2; 3; 4; 5 mg/L), Biuret Solution ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  and  $\text{KNaC}_4\text{H}_4\text{O}_6$ ), and 30% NaOH solution.

The stages of making single-cell protein include isolation of *S. cerevisiae*, making media for pineapple peel, making a starter, and the fermentation process.

#### 1. *S. cerevisiae* Isolation

Instant dry yeast containing *S. cerevisiae* with an additional 9 ml of sterile distilled water, then was diluted up to  $10^{-6}$ . To get a single colony, dilution was carried out so that the growth of *S. cerevisiae* is not too dense. The inoculum at  $10^{-1}$  dilution to  $10^{-6}$  dilution was cultured on PDA medium using the slant method, then two inoculums were evenly scratched on the surface, then incubated at  $30^\circ\text{C}$  for 48 hours (Febriyanti et al., 2017). Colony morphology of growing *S. cerevisiae* is identified macroscopically from the colony form such as pearl grains along the inoculation line, smooth surface texture, and yellowish-white color (Septriani, 2009).

#### 2. Pineapple Peel Wasted Medium Preparation

500 grams of pineapple peel waste was washed, then blended with 500 ml aquadest for 2 minutes with a blender speed of 21,000 rpm. The pineapple solution is filtered using a filter cloth. Pineapple filtrate is taken and pasteurized at  $61^\circ\text{C}$  for 30 minutes and then cooled (Pawignya, 2011). This solution is referred to as a fermentation medium.

#### 3. Preparation of *S. cerevisiae* Starter Solution

The starter solution contains purified *S. cerevisiae*. In making this starter, there are three treatments for adding carbon sources, namely: without adding a carbon source (control) and adding a carbon source in the form of fructose and a carbon source in the form of sucrose. The starter can be made on a source of 20 grams of fructose or sucrose carbon dissolved in 100ml of aquadest, the pH of the solution is adjusted to 5 by adding 1 N  $\text{CH}_3\text{COOH}$  solution, then adding 0.1 gram of nutrients ( $\text{NH}_2$ ) $_2\text{SO}_4$ , 0.1 gram of  $\text{K}_2\text{HPO}_4$ , 0,05 grams of  $\text{MgSO}_4$ , 0.01 grams of NaCl, and 0.01 grams of  $\text{CaCl}_2$  (Mondal et al., 2012). After that, the solution was sterilized at  $121^\circ\text{C}$  for 15 minutes. After a solution temperature of  $\pm 29^\circ\text{C}$ , the pure culture of *S. cerevisiae* that had been isolated was inserted in two ose needle colonies into the solution then fermented using a shaking incubator for 2 days at  $30^\circ\text{C}$  (Pawignya, 2011).

#### 4. Fermentation Process

135 ml of pineapple peel solution was added to the Erlenmeyer, after that the pH of the solution was adjusted to 4.5 with the addition of 1N  $\text{CH}_3\text{COOH}$  solution and then sterilized at  $121^\circ\text{C}$  for 15 minutes. After the solution temperature was  $\pm 29^\circ\text{C}$ , the solution was added with 15 ml of starter and fermented for 24 hours, 48 hours, and 72 hours (Pawignya, 2011).

#### 5. Test Method

##### a. Media pH Measurement (SNI-06-6989)

Measurement of the pH of the control media, fructose media, and sucrose media were carried out at 24, 48, and 72 hours using a pH meter that had been sterilized using a disinfectant.

##### b. Measurement of cell dry weight (Purwitasari, 2004)

50 ml of media samples centrifuged at 3000 rpm for 10 minutes. The precipitate is taken and transferred into a cup, then dried at  $60^\circ\text{C}$  for 5 hours. After drying, the cells are extracted by scraping them, then the resulting powder is weighed by the dry weight of the cells and analyzed for protein content.

##### c. Biuret method analysis of protein content (AOAC, 1995)

Protein content was measured by the biuret method using a UV-VIS spectrophotometer with a wavelength of 550nm.

This experiment was conducted by repeating the treatment twice and repeating the test analysis three times. The test analysis was carried out on single-cell proteins, namely pH, cell dry weight, and protein content. Data analysis was carried out using the test of variance at the 95% confidence level, for treatments that were significantly different, further tested the Duncan Multiple Range Test (DMRT) with a confidence level of 95%.

## RESULTS AND DISCUSSION

Single-cell protein is a product of dry cell or microorganism biomass that can be used as a source of protein for food and feed (Babazadeh et al., 2014). In this study, the microorganism used was *S. cerevisiae*. *S. cerevisiae* contains lots of vitamins, especially vitamin B complex and minerals, so it is very good to be consumed as a dietary supplement for humans and animals. Also, the percentage of *S. cerevisiae* protein content was 45% higher than soy protein by 35% (Masithoh & Nim, 2012).

The manufacture of single-cell protein in this study was carried out by adding a carbon source to the starter. The results of pH testing for each

treatment and fermentation time can be seen in Table 1.

Changes in pH during fermentation of 24, 48, and 72 hours were still in the range of *S. cerevisiae* growth, namely at 3.72-4.71, so that they did not inhibit the growth of *S. cerevisiae*. According to Pawignya (2011) stated that the pH of *S. cerevisiae* growth ranged from 3.5-5.5 with an incubation temperature of 25-30°C. Before fermentation, the pineapple peel waste media in this study was set at pH 4.5, because *S. cerevisiae* grew well at that pH. During the fermentation time of 24, 48, and 72 hours, the media that was not added with a carbon source (control) had the highest pH of the media than the media with added fructose and sucrose. This is because the control media only uses the carbon source in the pineapple skin, namely glucose as much as 10.8% (Dhanasekaran et al., 2011).

The addition of sucrose and fructose greatly affects the pH of the media, because this carbon is used by *S. cerevisiae* in the starter making, resulting in organic acids which lower the pH. Sucrose is a disaccharide consisting of glucose and fructose. To hydrolyze disaccharides into monosaccharides, *S. cerevisiae* uses invertase enzymes (Azizah et al., 2012). This hydrolysis process causes the pH of the media with the addition of sucrose carbon to be

higher than the addition of fructose carbon because *S. cerevisiae* needs to hydrolyze disaccharides for the fermentation process, whereas in the fructose medium *S. cerevisiae* does not need to hydrolyze it first. During fermentation, *S. cerevisiae* produces zymase and invertase enzymes, under an aerobic conditions the zymase enzyme will break down sucrose into monosaccharides (glucose and fructose) (Artiyani, 2011).

During fermentation of 0-24 hours, all media experienced a decrease in media pH. This is because during fermentation of *S. cerevisiae* it produces organic acids such as malic acid, citric acid, acetic acid, tartaric acid, lactic acid, butyric acid, and propionic acid as a by-product (Masithoh & Nim, 2012). *S. cerevisiae* has a generation time of 2 hours so that during 24 hours of fermentation, *S. cerevisiae* has undergone a process of cell addition and has entered a logarithmic growth phase (Artiyani, 2011). During 24 hours to 48 hours of fermentation, the pH decreased for each growth medium. The decrease in media pH from 24-48 hours is not as much as fermentation for 0-24 hours due to fermentation of 24-48 hours *S. cerevisiae* has entered a slow growth phase (Pratiwi, 2018). In this phase, the possibility of nutrients has decreased so that the pH decrease is not too much.

**Table 1.** Degree of medium acidity during fermentation

Carbon source	pH time hour		
	24	48	72
Control	4.34 ± 0.04 <sup>bc</sup>	4.27 ± 0.04 <sup>b</sup>	4.71 ± 0.01 <sup>c</sup>
Fructose	3.83 ± 0.16 <sup>a</sup>	3.72 ± 0.02 <sup>a</sup>	3.81 ± 0.01 <sup>a</sup>
Sucrose	4.22 ± 0.07 <sup>b</sup>	4.11 ± 0.00 <sup>ab</sup>	4.33 ± 0.02 <sup>bc</sup>

**Note:** Different letter notations indicate a significant difference in the confidence level of the DMRT test of 95%

**Table 2.** Cell dry weight (g) fermentation result

Carbon source	Cell dry weight (g) time hour		
	24	48	72
Control	0.2804 ± 0.03 <sup>b</sup>	0.2168 ± 0.01 <sup>a</sup>	0.2963 ± 0.00 <sup>c</sup>
Fructose	0.3757 ± 0.01 <sup>cd</sup>	0.4167 ± 0.05 <sup>d</sup>	0.4203 ± 0.01 <sup>d</sup>
Sucrose	0.3608 ± 0.01 <sup>c</sup>	0.3712 ± 0.02 <sup>cd</sup>	0.3385 ± 0.03 <sup>c</sup>

**Note:** Different letter notations indicate a significant difference in the confidence level of the DMRT test of 95%

Fermentation at 72 hours has increased pH. This is because the food and nutrients of *S.cerevisiae* are starting to run low so that they undergo a change in protein in the medium for its metabolic activity. This metabolic process causes the formation of metabolites resulting from protein degradation such as urea and ammonium ions which cause an increase in pH (Purwitasari et al., 2004). Research conducted by Purwitasari (2004) regarding the manufacture of single-cell proteins from coconut waste medium shows that the longer fermentation time of the pH medium increases. Also, research conducted by Hasibuan (2019) also stated that during fermentation of 0-24 hours the pH of the media has decreased, while during 48-72 the pH of the media has increased.

Cell dry weight is a method of calculating cells by separating the substrate and cells formed from the fermentation process. The separation process uses a centrifugation technique and the residue that settles is dried at a temperature of 60°C for 5 hours. The results of the cell dry weight test for each treatment and fermentation time can be seen in Table 2.

Based on Table 2, there is no specific trend from the results of the analysis of the dry weight of cells to the fermentation time. This occurs because the dry weight of impure cells comes from the cells resulting from the fermentation process. Several impurities are weighted as dry weight of cells, namely pineapple juice fibers which are filtered during filtering.

The increase in dry cell weight is strongly influenced by the rate of growth and the ability of cells to utilize nutrient sources in the media. The more nutrients *S.cerevisiae* needed for growth, the resulting increase in the number of cells during the incubation time (Masithoh & Nim, 2012). The incubation time did not give significantly different results, but the longer the incubation time, the dry weight of the cells increased but not too much. Apart from the rate of growth, the dry weight of cells is very much influenced by oxygen. Oxygen and aeration are very important for the growth of *S.*

*cerevisiae* because oxygen is quickly absorbed by *S. cerevisiae* and used to synthesize unsaturated fatty acids and sterols that form cell membranes, oxygen also stimulates the synthesis of molecules needed by *S. cerevisiae* to carry out metabolism and take up sugar. disaccharides. Aeration also has a very important role, according to Casselman (2005), continuous agitation/aeration can result in an increase in the number of yeast cells 10 to 15 times. This is evidenced by research conducted by Casselman (2005) that agitation with stable and sustainable stirring increases the number of yeast cells compared to the method of stirring. The unstable stirring factor also causes the cell growth process to have no specific tendency for each treatment.

Based on the analysis of completely randomized design data for cell dry weight, the addition of a carbon source has a very significant effect on cell dry weight. The fermentation time does not affect the dry weight of the cells. Based on the DMRT analysis, it is known that the fermentation time of *S.cerevisiae* on fructose and sucrose media was not significantly different, while in the control medium the fermentation time at 48 hours showed a significant difference in dry weight with fermentation time at 24 and 72 hours. In addition, the addition of carbon fructose and sucrose in the fermentation medium was significantly different from the control media, this is because the controlled media only used glucose contained in pineapple peels. The optimum dry weight of cells in the fermentation medium with the addition of fructose was 0.4203 grams, while the lowest cell dry weight in the controlled media was 0.2168 grams. This is similar to research conducted by Masithoh (2012), which shows that the addition of sucrose to single-cell protein-making from rice bran media produces more cell dry weight.

Analysis of protein content is one way to determine the absorption of nutrients during fermentation by *S.cerevisiae* cells. The results of testing protein levels in each treatment and fermentation time can be seen in Table 3.

**Table 3.** Protein content (mg/L) fermentation results

Carbon Source	Protein content (mg/L) time hour		
	24	48	72
Control	89.81 ± 15.18 <sup>b</sup>	73.03 ± 10.96 <sup>ab</sup>	107.8 ± 1.48 <sup>c</sup>
Fructose	85.05 ± 9.10 <sup>ab</sup>	72.37 ± 7.95 <sup>a</sup>	69.08 ± 0.11 <sup>a</sup>
Sucrose	89.66 ± 0.71 <sup>b</sup>	77.14 ± 8.00 <sup>ab</sup>	85.55 ± 0.82 <sup>ab</sup>

Based on Table 3, the cell protein content of fructose media tends to decrease during the fermentation time. Meanwhile, the levels of cell protein from sucrose and control media increased. Meanwhile, research results conducted by Pawignya (2011) states that the longer the fermentation time produces more cell protein levels. The increase in protein levels can be caused by the increase in yeast cells which act as single-cell protein agents because *S. cerevisiae* consists of 50-52% crude protein, 30-37% carbohydrates, 4-5% fat, and 7-8% minerals. (Reed & Nagodhawithana, 1988).

The single-cell protein content produced in the Purwitasari (2004) study was 26-38%. The longer the fermentation takes, the less protein content in the Purwitasari (2004) study. Also, the protein content produced in Mashitoh's (2012) study regarding the manufacture of single-cell proteins from bran waste was 29-37%. Meanwhile, research conducted by Hasibuan (2019) regarding the manufacture of single-cell proteins from rice water waste and pineapple waste is 0.02%. Then, research conducted by Widanti (2012) regarding the manufacture of single-cell protein from tofu liquid waste has a protein cell content of 0.6%. Meanwhile, the optimum protein content in this study was 0.01%.

Based on the calculation of complete randomized design data analysis of protein content, the addition of carbon sources has a significant effect on cell protein levels. The fermentation time also affects the levels of cell protein significantly. DMRT further test analysis showed that the cell protein levels between the control media, fructose, and sucrose were not significantly different. However, the protein content of the 72-hour fermentation control medium was different from other media. The optimum protein content in the controlled media was 107.8 mg/L, while the lowest protein content in the fructose medium was 69.08 mg/L. This is because the media with added carbon contains 30.8% sugar, this causes *S.cerevisiae* to experience osmotic pressure. According to Wardani et al. (2013) stated that fermentation in a medium containing 25% sugar is classified as fermentation under conditions of high osmotic pressure so that cells experience lysis due to hypertonic medium causing disrupted cell metabolism.

## CONCLUSION

The addition of carbon fructose and sucrose to the starter of single cell protein production affected

the pH of the fermentation medium and the dry weight of the cells. The addition of carbon has no effect on protein content. The carbon source in the form of fructose can increase the dry weight of the cells produced, while sucrose can increase the protein content of the resulting dry cells.

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