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Enhancing Single Cell Protein Production of *Saccharomyces cerevisiae* Using Soybean Hull Waste with Dextrose Variations

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Abstract *– Single Cell Protein (SCP) is a microprotein derived from the dry biomass of non-pathogenic microorganisms and offers a promising alternative protein source for both humans and animals. One of the advantages of SCP production is its potential to utilize organic waste as a growth medium, thereby contributing to environmental sustainability. Soybean hull waste has been identified as a nutrient-rich substrate capable of supporting the growth of various microorganisms used in SCP production. This study aimed to optimize the growth medium for S. cerevisiae by utilizing soybean hull waste supplemented with varying concentrations of dextrose, in order to determine the most effective dextrose level for SCP production. The experiment involved cultivating S. cerevisiae in filtrates of soybean hull waste supplemented with five different dextrose concentrations: 0% (FD0), 1% (FD1), 2% (FD2), 3% (FD3), and 4% (FD4), as well as a standard Yeast Potato Dextrose (YPD) medium used as a control. Fermentation was initiated with an inoculum concentration of* 1.3×10^6 *CFU/mL across all treatments then incubated at 28–30°C for 72 hours. Biomass, moisture content and protein content of yield were measured at the end of the fermentation. The results showed the dextrose conc. significantly affected pH levels, biomass yield, moisture, and protein content of the final product. Higher dextrose conc. generally led to better yield. Among the treatments, FD4 (4% dextrose) produced the highest biomass (0.091 g/10 mL) and protein content (18.43%). These finding showed that soybean hull waste can serve as a sustainable growth medium for the S. cerevisiae-SCP production, with 4% dextrose supplementation yielding the most optimal results.*

Keywords: *dextrose, fermentation, medium, single cell protein (SCP), soybean hull*

INTRODUCTION

Single-Cell Protein (SCP) refers to microorganism based bioprotein (microprotein). It is a dried biomass product derived from non-pathogenic microorganisms, such as microalgae, yeast or bacteria, that are rich in protein (Sharif *et al.*, 2021). The protein content of SCP typically ranges from 30% to 85% of its dry biomass weight (Li *et al.*, 2024; Raziq *et al.*, 2020). SCP also contains essential amino acids (e.g., methionine and lysine), carbohydrates, fatty acids, nucleic acids, minerals, and vitamins, making it highly nutritious and promising alternative protein source that could replace or complement to conventional protein sources from fisheries, livestock, and agriculture (Maryana *et al.*, 2016; Raziq *et al.*, 2020; Azwar *et al.*, 2021; Koukomaki *et al.*, 2024). SCP production offers several advantages over plant and animal-based protein source. It requiring neither large areas nor significant water storage. It is independent of seasonal changes and allows continuous year-round production with high biomass yield in a short time (Sharif *et al.*, 2021). SCP can be consumed directly as a supplement or incorporated into food product. Instead of for fulfilling protein intake requirements, SCP also serves as a natural antibiotic by producing antimicrobial compounds such as enzymes or metabolites that inhibit pathogens or even compete with them for nutrients in the digestive tract (Raziq *et al.*, 2020).

Microorganisms used for SCP production must non-pathogenic or non-toxic that commonly are generally classified as GRAS (Generally Recognized as Safe). *Saccharomyces cerevisiae* is one such organism, and recognize to capable of producing SCP in substantial quantities (Raziq *et al.*, 2020; Azwar *et al.*, 2021; Do *et al.*, 2024). This yeast is widely utilized as animal feed, a fermentation agent for foods and beverages, as well as bioethanol (Kustyawati, 2018; Khazalina, 2020; Parapouli *et al.*, 2020). The growth media commonly used in *S. cerevisiae* SCP production include Yeast Potato Dextrose (YPD) or Yeast Extract Peptone Dextrose (YEPD), and Yeast Extract Peptone Glycerol (YEPG). However, SCP production can also utilize various fermentation media, including organic waste-based substrates such as agricultural waste, dairy processing waste, fruit peels, molasses, and even paper or lignoselulose based-waste (Gervasi *et al.*, 2017; Thiviya *et al.*, 2022; Koukomaki *et al.*, 2024). Organic waste rich in nitrogen and carbon can serve as a fermentation substrate for SCP production (Maryana *et al.*, 2016; Harahap *et al.*, 2025).

Soybean hulls are an organic waste from soybean-based food industries such as tempeh, tofu, soy sauce, and soy milk production. This waste contains several valuable nutrients, such as 24.84% crude fiber, 17.98% protein, and 5.5% fat, which serve as essential carbon and nitrogen sources (Jariyah *et al.*, 2022), making them a potential carbon and nitrogen source for *S. cerevisiae* cultivation. To enhance SCP production, additional carbon sources like monosaccharides (e.g., glucose, dextrose) or disaccharides (e.g., sucrose) may be added (Nurmalasari & Maharani, 2020). Glucose play a crucial role as the primary biomolecule for energy production, which is essential for microbial growth. The glucose concentration in the growth medium influences the growth and yield of SCP, including its protein content. Glucose (L-glucose) exists in another form known as dextrose (D-glucose), which is the most commonly used carbon source in microbial growth media. Therefore, this study aims to investigate the effect of dextrose supplementation in the growth medium for *S. cerevisiae* SCP production using soybean hull waste as a substrate, compared to the standard YPD medium. The dextrose concentration will be varied to determine the optimal dextrose concentration for maximizing *S. cerevisiae* biomass production based on SNI 01-4136-1993 standards.

MATERIALS AND METHODS

This study employed an experimental method using a Completely Randomized Design (CRD) with a single factor: variations in dextrose concentration (conc.). The experiment included four treatments with dextrose concs. of 0%, 1%, 2%, 3%, and 4%. YPD medium was used as a control medium.

1. Research Samples

The pure culture of *S. cerevisiae* used in this study was obtained from Agavilab (Dago, Bandung, West Java). Meanwhile, soybean hull waste was collected from a tempeh factory in Cibiru, Bandung, West Java, Indonesia.

2. Preparation of SCP Production Medium

2.1. FD (Filtrate + Dextrose) Medium

FD medium was prepared using soybean hull waste filtrate supplemented with dextrose. A total of 60 g of soybean hull waste was hydrolyzed with 100 mL of 10% HCl and heated at 100°C for one hour in a water bath. The extract was then cooled and filtered using Whatman No. 1 filter paper. Subsequently, 2 g of $(NH_4)_2SO_4$, 1 g of KH_2PO_4, 0.5 g of $MgSO_4.7H_2O$, 0.1 g of NaCl, and 0.1 g of $CaCl_2$ were added to 1 L of filtrate. The FD medium was then supplemented with dextrose at concentrations according to the treatments (Table 1) and homogenized (Sharma & Kanikasharma, 2017).

Table 1. Dextrose Composition in Soybean Hull Waste Filtrate Medium

Treatment	Dextrose Amount (g/100 mL soybean hull waste medium)
FD0	0
FD1	1
FD2	2
FD3	3
FD4	4

Note: FD0: 0% of dextrose, FD1: 1% dextrose, FD2: 2% dextrose, FD3: 3% dextrose dan FD4: 4% of dextrose

2.2. YPD (Yeast Potato Dextrose) Medium as a Control (K)

YPD medium was prepared by dissolving 2 g of peptone, 1 g of yeast extract, and 2 g of dextrose in 100 mL of distilled water. The solution was homogenized and heated using a magnetic stirrer. Both FD and YPD media were then sterilized at 121°C for 15 minutes using an autoclave.

3. Preparation of *S. cerevisiae* Suspension

The *S. cerevisiae* suspension was prepared using a dilution method, and yeast cell density was determined using the Total Plate Count (TPC) method. Initially, *S. cerevisiae* was revitalized on PDA medium at 28°C for 48 hours. A loopful of the revitalized inoculum was then homogenized in a test tube containing 9 mL of sterile distilled water. A 1 mL aliquot of the initial suspension was diluted in 9 mL of sterile distilled water, labeled as a 10^{-1} dilution. This dilution process was repeated up to 10^{-6}. From each dilution (10^{-1} to 10^{-6}), 1 mL was inoculated onto PDA medium using the pour plate technique. Each sample was plated in duplicate and incubated at 28°C for 48 hours. The resulting colonies were counted using a colony counter, and yeast cell density was calculated using the following TPC formula (Kirana *et al.*, 2023):

$$Microbial\ Count\ (CFU/mL) = Number\ of\ Colonies\ \times \frac{1}{Dilution\ Factor}$$

4. Production of *S. cerevisiae* Single Cell Protein via Fermentation

A total of 2 mL of *S. cerevisiae* suspension with a cell density of 10^6–10^7 CFU/mL (determined from TPC results) was aseptically inoculated into each medium. Fermentation was conducted at 28–30°C in a shaker incubator at 120 rpm for 72 hours. The pH was measured before and after fermentation using a pH meter (Sharma & Kanikasharma, 2017).

5. Measurement and Data Collection

All fermentation samples were collected and centrifuged at 3,000 rcf for 15 minutes. The supernatant was discarded, and the remaining pell*et al*ong with the Eppendorf tube was weighed to determine wet biomass. The biomass was then washed with 10 mL of distilled water and centrifuged again (Anggrayeni & Kusdiyantini, 2019).

5.1. Determination of Dry SCP Biomass

The pellet was dried at 60°C for 72 hours in an oven. Once dried, the sample was weighed, and biomass weight was calculated using the following formula (Aldila, 2022):

$$B = B2 - B1$$

Note:

B : biomass (g)
B2 : weight of the tube containing biomass (g)
B1 : weight of the empty tube (g)

The dried sample was then ground using a mortar and pestle to obtain a fine cell powder for protein analysis.

5.2. Moisture Content Calculation

The moisture content of each sample was determined using the following formula (Danuwewra *et al.*, 2021):

$$Moisture\ Content\ (\%) = \frac{Initial\ Weigth - Final\ Weight}{Initial\ Weight} \times 100\%$$

5.3. BSA Curve Standard Preparation

Before analyzing the protein content of the SCP, a standard BSA (Bovine Serum Albumin) curve was prepared. BSA solutions with concentrations of 0.06, 0.12, 0.18, 0.24, and 0.3 mg/mL were prepared. Each solution was mixed with 5 mL of Lowry reagent C and homogenized using a vortex mixer. Then, 0.5 mL of Lowry reagent E was added, and the solution was incubated at room temperature for 30 minutes. Absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 590 nm. The optical density (OD) values obtained were used to construct a linear standard curve for SCP protein analysis (Sherly *et al.*, 2023).

5.4. Protein Content

A total of 0.5 g of SCP powder was dissolved in 100 mL of distilled water, filtered, and diluted again with an additional 100 mL of distilled water to form the sample solution. Then, 1 mL of the sample solution was mixed with 1 mL of Lowry reagent D, homogenized, and incubated at room temperature for 5 minutes. Next, 3 mL of Lowry reagent E was added, mixed, and incubated at room temperature for 45 minutes. Finally, 1 mL of the sample was transferred to a cuvette, and absorbance was measured at 590 nm using a UV-Vis spectrophotometer (Sherly *et al.*, 2023). The protein concentration was then calculated using the linear equation derived from the BSA standard curve. The protein content (%) is then calculated using the following formula:

$$Protein\ content\ (\%) = \frac{C \times FP \times V}{BS} \times 100\%$$

Note:

C : Concentration
FP : Dilution Factor
V : Volume of added water (mL)
BS : Sample weight (g)

6. Data Analysis

The pH data of the medium before and after fermentation were analyzed using Wilcoxon test (non-parametric). Other data were analyzed using a Kruskal-Wallis. For treatments showed significantly different results, a post-hoc test was conducted as appropriate. Additionally, the data were also analyzed descriptively by comparing them with the Indonesian National Standard (SNI) 01-4136-1993.

RESULT AND DISCUSSION

1. Yeast Count in Suspension of *S. cerevisiae*

Six serial dilutions of *S. cerevisiae* suspensions were prepared in this study (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}). The yeast count in each suspension sample was determined using the Total Plate Count (TPC) method and the results are presented in Table 2.

Table 2. TPC result of S. cerevisiae suspension preparation

Dilution	Replication		CFU/ml
	1	2	
10^{-1}	TNTC	TNTC	TNTC
10^{-2}	TNTC	TNTC	TNTC
10^{-3}	TNTC	TNTC	TNTC
10^{-4}	134	137	$1{,}3\times10^{6}$
10^{-5}	14	19	TFTC
10^{-6}	4	2	TFTC

Note: TNTC (Too Numerous To Count) and TFTC (Too Few To Count)

Based on the result, it is known that the most suitable suspension for PST production is at the fourth dilution. This is because, in general, the microbial density used in PST production ranges from 10^{6}-10^{7} CFU/mL (Sharma & Kanikasharma, 2017). Microbial conc. is directly affect the fermentations yield. If the microbial conc. is too low, fermentation will proceed slowly then resulting in low yields. In contrast, higher microbial conc. in the medium lead to higher fermentation yields (Aldila, 2022). However, excessively high microbial conc. may cause nutrient competition among the microbes and slowing their growth. This condition may also induce sporulation, where a portion of the energy is allocated for survival rather than cell proliferation, ultimately reducing the overall yield.

2. pH of the Fermentation Medium

pH is a critical environmental factor influenced by microbial growth and activity. pH represents the concentration of hydrogen ions in a sample and is expressed as -log $[H^{+}]$, which is the negative logarithm of the hydrogen ion or proton concentration (Sajin et al., 2020). The Wilcoxon test results showed a p-value of 0.122 ($p \geq 0.05$), indicating that the pH before and after fermentation was not significantly different. Although the difference was not statistically significant, changes in pH were still observed between the beginning and end of the incubation period across all growth media treatments (Table 3). Fluctuations in pH during fermentation serve as an indicator that the fermentation or cultivation process is proceeding effectively. The initial pH of all treatment media corresponds to the optimal pH for the growth of *S. cerevisiae*. In general, *S. cerevisiae* is classified as an acidophilic microorganism, meaning it thrives in acidic environments. The optimal pH range for yeast growth varies between pH 4 and 6, depending on temperature, oxygen availability, and yeast strain (Narendranath et al., 2005).

Table 3. pH levels of the medium before and after fermentation

Treatment	pH	
	Before Fermentation	After Fermentation
K	6,2±0,2499	7,0±0,1266
FD0	5,5±0,0058	7,2±0,1706
FD1	5,5±0,0058	7,3±0,0586
FD2	5,5±0,010	6,3±0,2326
FD3	5,5±0,0058	4,6±0,2851
FD4	5,5±0,0153	4,2±0,1656

Note: FD0: 0% of dextrose, FD1: 1% dextrose, FD2: 2% dextrose, FD3: 3% dextrose dan FD4: 4% of dextrose

As shown in Table 3, the pH of the FD0, FD1, FD2, and K media tended to increase at the end of the fermentation period (after 72 hours). This increase may be attributed to the presence of metabolites such as urea and ammonium ions in the medium. The depletion of carbon sources over time prompts yeast to utilize alternative energy sources, such as proteins. The degradation of proteins produces metabolites like urea and ammonium ions, which can contribute to an increase in pH (Nurmalasari & Maharani, 2020). In contrast, the FD3 and FD4 treatments exhibited a decrease in pH after fermentation. These media contained relatively high dextrose concentrations, suggesting that dextrose was still present in the medium after 72 hours, allowing yeast to continue utilizing it for growth. Additionally, according to Azizah et al. (2012) and Malau et al. (2022), a decrease in pH can result from CO_2, a byproduct of yeast metabolism. When CO_2 dissolves in liquid media, it forms carbonic acid (H_2CO_3), which subsequently dissociates into hydrogen ions (H^+), leading to a reduction in pH. The balance between H^+ and OH^- ions influences pH, with an excess of H^+ ions rendering the solution acidic, while an excess of OH^- ions results in a basic solution. Furthermore, Kustyawati (2018) stated that during fermentation, *S. cerevisiae* produces various metabolic products, such as pyruvic acid, acetic acid, and ethyl acetate. These metabolites also contribute to the reduction in pH in the fermentation medium.

3. Dry Biomass of *S. cerevisiae* SCP

The dry biomass of *S. cerevisiae* yielded from all treatments is detailed in Figure 1. The Kruskal-Wallis analysis of the biomass data yielded a p-value of 0.567, indicating no significant differences among the treatments ($p \geq 0.05$). But, it can be seen that FD4 (soybean hull filtrate medium with 4% dextrose) produced the highest biomass (0.091 g), followed by FD3 (0.090 g), FD2 (0.082 g), K (0.077 g), FD1 (0.070 g), and FD0 (0.066 g) as the lowest. Despite the differences were not statistically significant (p-value of 0.567), these result indicate that soybean hull filtrate medium supplemented with appropriate dextrose conc. can enhance SCP biomass production compared to the standart YPD medium. Moreover, the findings suggest that dextrose conc. plays a role in optimizing SCP production by *S. cerevisiae.*

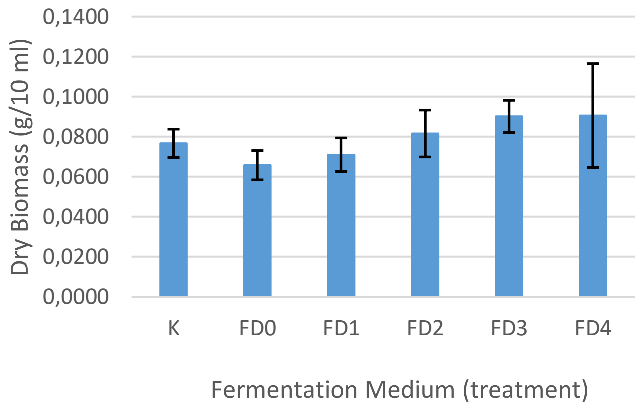

Figure 1. Average cell biomass of *S. cerevisiae* produced at the end of fermentation from each growth medium

The addition of dextrose can increase the carbon source in the medium, thereby promoting better microbial growth and resulting in higher biomass production. The higher biomass yield in FD4 compared to other treatments may be due to the dextrose conc. (4%), that serves as the primary energy source for microbial growth and replication. Higher nutrient availability leads to greater energy production, resulting in increased biomass yield (Prastujati et al., 2022).

4. Moisture Content of *S. cerevisiae* SCP

Moisture content refers to the total water present in a sample including food product. Moisture analysis is a critical parameter in the food industry, as it determines the quality and shelf stability of food products by assessing their susceptibility to deterioration (Daud et al., 2019). Moisture content affects the physical and chemical properties of food during storage and processing, ultimately influencing product quality (Dunuweera et al., 2021). Therefore, moisture content analysis was conducted on SCP to evaluate its potential as an alternative food source. The results of the moisture content analysis for *S. cerevisiae* biomass are presented in Figure 2.

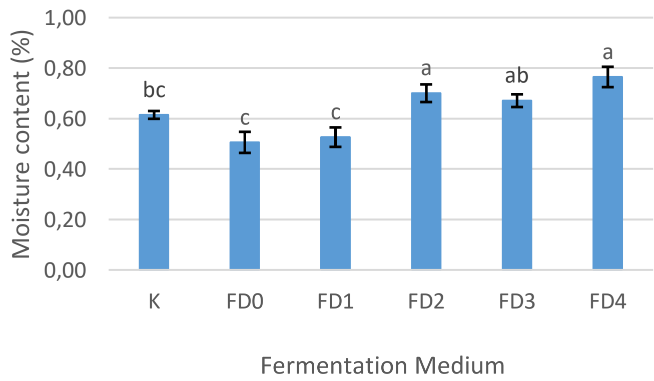

Figure 2. The moisture content of *S. cerevisiae* biomass in each growth medium

Variations in moisture content were observed across treatments, and the Kruskal-Wallis test on the moisture content of *S. cerevisiae* cell biomass showed a p-value of 0.015 ($p < 0.05$), indicating a statistically significant difference. Therefore, each treatment was grouped into specific subsets based on the notations shown in Figure 2. The highest moisture content was found in FD4 (0.76%), followed by FD2 (0.70%), FD3 (0.67%), K (0.61%), FD1 (0.53%), and the lowest in FD0 (0.51%). However, based on its value, the biomass of PST in all treatments has a moisture content of less than 10%, meaning it meets the standard moisture content for PST. PST must be dried until its moisture content reaches around 10% or be condensed and acidified to prevent spoilage (Ghorai et al., 2009).

5. Protein Content of *S. cerevisiae* SCP

The protein content of *S. cerevisiae* SCP was analyzed using the Lowry method which is considered one of the most accurate for quantitatively determining protein content, with a sensitivity of up to 0.01 mg/mL. In this method, a standard protein solution is required to make equation to determine the protein concentration and to calculate the total protein content of the SCP samples. Bovine Serum Albumin (BSA) was used as the standard, prepared at five different concentrations (0.06, 0.12, 0.18, 0.24, and 0.30 mg/mL) to construct a standard curve (Figure 3) and resulting a linear regression equation: y= 1,6158x + 0,0058.

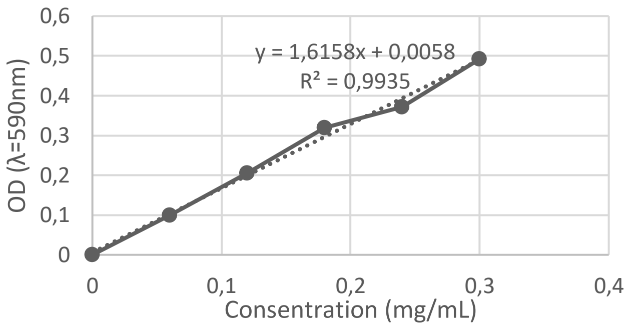

Figure 3. Standard curve of Bovine Serum Albumin (BSA)

The results of the protein conc. and protein content measurement for PST *S. cerevisiae* in this study are presented in Table 4. The Kruskal-Wallis test showed that all treatments had p-values ≤ 0.05, indicating statistically significant differences among them. Therefore, based on the results of the post hoc test, each treatment was significantly different from the others and was classified into separate subsets, as indicated by the notations in Table 4. The highest protein content was observed in the FD4 (18,43%), while the lowest protein content was found in FD0 (1,08%). In general, the protein content of SCP is related to the nutrients present in the medium. Soybean hull waste is known for its high of protein and nitrogen content, making it an ideal medium for SCP production. Organic waste that is rich in nitrogen and carbon can serve as an effective substrate for fermentation in SCP production (Maryana et al., 2016; Harahap et al., 2024). The result of this study demonstrate that protein content in the SCP increases with higher dextrose conc. in the soybean hull treatment medium, suggesting that the addition of dextrose positively influences the protein content of the SCP produced.

Tabel 4. Protein content of S. cerevisiae SCP in each medium

Perlakuan	Protein Cons (mg/mL)	Protein content (%)
K	0,023	$9{,}09\pm0{,}00006^{b}$
FD0	0,003	$1{,}08\pm0{,}00003^{f}$
FD1	0,003	$1{,}28\pm0{,}00003^{e}$
FD2	0,004	$1{,}45\pm0{,}00003^{d}$
FD3	0,006	$2{,}52\pm0{,}00003^{c}$
FD4	0,046	$18{,}43\pm0{,}00023^{a}$

Moreover, the protein content of SCP is also known to correlate with the amount of biomass produced. This aligns with the statement by Reed and Nagodhawithana (1988), as cited in Nurmalasari & Maharani (2020), which explains that the high protein content in SCP can be attributed to an increased number of microbial cells. In general, the greater biomass typically correlate to higher protein content. Microorganisms have a much higher protein content of 30–70% in the dry mass (Garimela et al., 2017). Then microbial biomass production is strongly influenced by the nutrient content of the growth medium. The nutrient availability plays a crucial role in determining the rate of microbial growth. An optimally nutrient-rich medium promotes better microbial proliferation, leading to increased biomass accumulation and elevated protein content (Azwar et al., 2021). This trend was evident in the current study, which is the FD4 treatment not only exhibited the highest protein content but also produced the greatest biomass of S. cerevisiae. These findings suggest that FD4 provided the most favorable nutritional conditions for *S. cerevisiae* growth among all treatments.

Interestingly, the protein content in FD3 and the control treatment did not align proportionally with their biomass levels. Despite FD3 producing relatively high biomass, its protein content was lower than that of the control, which had a less biomass yield. This inconsistency may be due to the earlier onset of the stationary phase in FD3 compared to FD4 and the control, under identical fermentation conditions and period. The stationary phase is typically characterized by a depletion of nutrients or substrates in the medium, which can reduce protein synthesis and may lead to protein degradation as a cellular response for cell's survival mechanisms (Napitulu et al., 2024). Therefore, further research is needed to observe the growth curve of *S. cerevisiae* in each fermentation medium. In addition, the carbon-to-nitrogen ratio in the medium also influences protein production. It is suspected that the ratio between the carbon source and the nitrogen content present in soybean seed hull in FD3 may not be suitable for *S. cerevisiae* protein production. Furthermore, it has been reported that the optimal carbon-to-nitrogen ratio ranges from 5:1 to 8:1 (Raita et al., 2022).

Tabel 5. SCP production results of S. cerevisiae using soybean hull waste with varying dextrose concentrations compared to the Indonesian National Standard (SNI 01-4136-1993).

Treatment	Parameters		
	Biomass (g/10 ml)	Moisture content (%)	Proteint content (% b/b)
K	0,077±0,0071	0,61±0,0155^{bc}	9,09±0,00006^{b*}
FD0	0,066±0,0073	0,51±0,0417^{c}	1,08±0,00003^{f*}
FD1	0,070±0,0084	0,53±0,0385^{c}	1,28±0,00003^{e*}
FD2	0,082±0,0117	0,70±0,0349^{a}	1,45±0,00003^{d*}
FD3	0,090±0,0080	0,67±0,0252^{ab}	2,52±0,00003^{c*}
FD4	0,091±0,0259	0,76±0,0404^{a}	18,43±0,00023^{a*}
SNI 01-3136-1993	-	**Maks 10**	**Min 40**

Note: - (There is no standard); *(Do not meet the standart); highlight (highest result)

Based on the Indonesian National Standard (SNI 01-4136-1993), the *S. cerevisiae* SCP produced from all treatment did not meet the standard requirements for use as animal feed (Table 5) as their protein content was below 40%. According to (Ramli et al., 2004), the protein content of SCP typically ranges from 44% to 65%, and can even reach up to 85% in some cases (Raziq et al., 2020)]. Nevertheless, the soybean hull waste medium can still be utilized for SCP production, as it supports yeast growth. However, further optimization is required to enhance its protein content.

CONCLUSION

This study demonstrates that soybean seed hull waste has potential as a fermentation medium for the production of *Saccharomyces cerevisiae* single-cell protein (SCP) although it has not yet met the protein content standard for animal feed. The optimal result was obtained using a filtrate-based soybean hull waste medium supplemented with 4% dextrose (FD4), which yielded the highest biomass of *S. cerevisiae* (0.091 g) and an exceptionally high protein content of 18.43%.

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