# β-Glucan production of *Saccharomyces cerevisiae* by using mango-fermented wastewater

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**Abstract** Three types of fermented mango wastewater including salted fermented, sweet fermented, and dried pickled, were used in this study. The result revealed that salted-fermented showed significantly highest in lightness (L*), while greenness (a*) and yellowness (b*) were found in sweet fermented. For chemical properties, all fermented wastewater showed acidity properties and significantly highest total soluble solids found in sweet-fermented. Microbiological properties were significantly highest in salted fermented. For the optimum condition formula for the growth of *Saccharomyces cerevisiae*, β-glucan production, and the ratio of wastewater to distilled water 1:4 showed the highest yield and β-glucan production. Then, this condition was selected to study the optimum time on yield and β-glucan production. Results exhibited that the significantly highest yield was in sweet fermented for yeast cultivation at 72 h. The significantly highest β-glucan production was also found in sweet fermented for yeast cultivation at 24 h. Overall, the obtained results implied that the wastewater from fermented mango could be used as the media for yeast cultivation and recycling of agro-industrial wastewater.

**Keywords:** *Saccharomyces cerevisiae*, Fermented mango wastewater, Polysaccharide, β-glucan

## Introduction

β-Glucan is known as a polysaccharide that is composed of glucose units linked together with glycosidic bonds and used in many industries, such as the pharmaceutical, food, feed, and cosmetics industries (Klis *et al*., 2002; Thomas *et al*., 2022). This substance also exhibits medicinal properties such as antimicrobial, antitumor antioxidant activities, and stimulation of the immune response. β -Glucancan be produced and extracted from different organisms,

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including plants, fungi, bacteria, and algae (Abdeshahian *et al*., 2021; Sengul and Ufuk, 2022). One species of fungi, *S. cerevisiae,* has been considered a potential industrial source of β-glucan production. Since the β-glucan from this yeast has various properties that are preferable to those found in other sources (Nya and Etukudo, 2023). Interestingly, β -glucan from *S. cerevisiae*is categorized as Generally Recognized as Safe (GRAS) by the FDA (Food and Drug Administration) (Leentjens *et al*., 2014). Moreover, this yeast can be rapidly and easily grown in a diverse array of culture media, at a low production cost, and its whole genome is already known (Mongkontanawat *et al*., 2018). Thus, *S. cerevisiae* is a good natural choice for β-glucan production. In addition, the β -glucan has been made up about 55- 65% w/w of yeast cell walls, consisting of both long chains of β -1,3-glucan (about 85% of the total) and short chains of β-1,6-glucan (Klis *et al*., 2002).

However, in terms of improving β -glucan production and reducing environmental pollution, the waste from various industries has been investigated for β-glucan production. For instance, Varelas *et al.* (2016) studied the utilization of winery yeast waste biomass to produce β-glucan to add value products as a part of an intergraded and sustainable wine industry. Additionally, Krisdaphong *et al.* (2018) were reported to extract and purify β-glucan from waste molasses yeast (*S. cerevisiae*) available from the ethanol industry. The β-glucan obtained was affected to suppress TNF-α and IL-6 production at 6 and 24 h. Furthermore, the β-glucan also indicated the potential to be used in the clinical evaluation. Our previous research evaluated the β-glucan production of *S. cerevisiae* using malva nut juice production wastewater. We exhibited that significantly higher %yield and β-glucan production was found in *S. cerevisiae* TISTR 5919 (0.67 ± 0.01 %w/v and 15.01 %w/w; respectively) than that *S. cerevisiae* Angel® and *S. cerevisiae* TISTR 5020 (0.39±0.01 %w/v, 12.69±2.62 %w/w and 0.41±0.03 %w/v, 8.33±1.26 %w/w; respectively) (Mongkontanawat *et al*., 2018). Utama *et al.* (2020) evaluated to determine the mass and antioxidant activity of ß-glucan extracted from *S. cerevisieae,* which was grown on vegetable and fruit wastes. This experiment evaluated three treatments banana waste, papaya waste, and napa cabbage waste as fermentation medium. The results showed that papaya waste was the best medium for producing ß-glucan, which resulted in 19.094 g of ß-glucan. Also, Bzducha-Wrobel *et al.* (2020) reported the production of β-glucan from *Candida utilis* strain on a biofermentor scale. The examined preparations' infrared spectra demonstrated a similar pattern to the β-glucan standard of *S. cerevisiae* origin. Moreover, Dewi *et al.* (2021) reported that tofu waste was used as the media for β-glucan production from *S. cerevisiae*. They found that P1 formulation is the best treatment to produce crude β-glucan.

In Thailand, the industry will rise, therefore, the waste from many industries will also increase (Chiemchaisri *et al*., 2007). For mango production, global production reached 41 million tons in 2020, from which 0.5% is industrialized as juices, jams, jellies, canned slices, mango leather, frozen chunks and slices pickles, chutney, and mango powder. During mango processing, 35-60% of the fruit is discarded, in many cases without treatment, generating environmental problems and economic losses. The by-products of the industrial processes are peel and seed, reaching 123,000 tons annually. In addition, the by-products from the fruit industry are expected to grow from 2.1% to 2.6 % in the next year (García-Mahecha *et al*., 2023). Therefore, there is much research has been reported on reusing and recycling the waste from this industry. Yunchalad *et al.* (2002) reported that a preliminary study for alleviating pollution from mango processing brine was conducted by recycling spent brines. Spent brines were reclaimed by sand filtration and an activated carbon system. Pereira *et al.* (2019) reported evaluating the lipase production by *Yarrowia lipolytica* from the wastes from industrial processing (mango seed and peel). Additionally, Hasanin *et al*. (2023) studied to determine the bacterial cellulose production by *Achromobacter* sp. using mango peel waste.

The best to reduce environmental pollution from the mango pickled industry wastewater, β-Glucan production of *S. cerevisiae* using mango fermented wastewater has been evaluated to find a new fermentation medium and recycling wastewater. In addition, no research on β-glucan production of *S. cerevisiae* using mango-fermented wastewater has been reported. Therefore, the aim of this study was to evaluate optimum conditions of yield and β-glucan content from three types of fermented mango wastewater, and the best formulation was selected to study the effect of fermented mango wastewater on yeast cell and β-glucan content production.

## Materials and methods

### Materials

Three types of fermented mango wastewater such as salt fermented, sweet fermented, and dried pickled were obtained from the Woraporn Limited company, Chachoensao province, Thailand. *S. cerevisiae* TISIR 5059 waspurchased from the Thailand Institute of Scientific and Technological Research (TISTR) in Pathum Thani province, Thailand. This yeast was cultured in YPD medium (1%w/v yeast extract,2 %w/v peptone, and 2 %w/v dextrose) at pH 4, 4 °C in a refrigerator for 48 h and subcultured every two months (Mongkontanawat *et al*., 2018).

*Fermented mango wastewater properties determination*

All fermented mango wastewater properties including physical, chemical, and microbiological properties were determined. For physical, properties, the color parameters were assessed by using a color meter (Nippon Denshoku, ZE-2000, Japan). The equipment was calibrated with a standard plate. Color measurements were expressed in L* indicating the lightness on a 0 to 100 scale from black to white; a* (+, -) indicating the redness or greenness, respectively; b* (+, -) indicating yellowness and blueness, respectively. The turbidity was estimated by using a spectrometer at wavenumber 860 mn. Three types of fermented mango wastewater were analyzed for the chemical properties' determination: pH, total soluble solid, salt content, and titratable acidity. Those chemical parameters were estimated by using a pH meter (Subtex, Taiwan), hand refractometer, Mohr method, and AOAC (2005); respectively. Viable cells count and yeast and mold count were performed for microbiological properties determination. The samples were cultured in PCA (Plate Count Agar) and PDA (Potato Dextrose Agar) medium at 30 °C for 48 h; respectively.

*Effect of ratio between fermented mango wastewater on yeast growth and β-glucan production*

In the first step, a fermentation medium was prepared by mixing wastewater with water invarious ratios of 1:0, 1:1, 1:2, 1:3, and 1:4 from three types of fermented mango wastewater. Yeast extract, peptone, and dextrose were combined with solution mediums in a 150 ml volumetric flask. The concentrations used were 1%, 2%, and 2% w/v at pH 4. Then, all mediums were sterilized by using an autoclave at 121 °C for 15 min. Yeast starter culture with 24 h cultivation ($10^8$ CFU/ml) 10 ml were added and then cultured in an incubatorshaker at 30 °C for 48 h. The yeast cells were collected by using centrifugation at 7,500 rpm for 10 min. Next, the sediment cells were freeze-dried, and the cell dry weight and β-glucan content were determined.

*β-Glucan content determination*

The β-glucan contents were determined by using a β-Glucan Assay Kit (Megazyme International, Wicklow, Ireland). The principle of the mushroom and yeast β-glucan assay kit is based on the determination of total glucan, which consists of α-glucan and β-glucan linkages. The bond of (1-3,1-6)-β-D-glucan, (1-3)-β-glucan, and α-glucan were dissolved and cut by concentrate hydrochloric acid at 100 °C for 2 h, and then the solution was incubated with

exo-1, 3-β-glucanase and β-glucosidase to get complete D-glucose for analysis of total glucan content. For α-glucan, it was digested to glucose with amyl glucosidase plus invertase, using a GOPOD reagent to measure glucose content. Finally, β-glucan content was calculated from total-glucan minus α-glucan (Magazyme, 2023, Mongkontanawat *et al.*, 2018)

### *Effect of cultivation time on yeast growth and β-glucan content production*

Second step, the best formulation wastewater medium was chosen for determining cultivation time on yeast growth, β-glucan production, yeast and mold count, and microstructure of yeast cells by using a Scanning Electron Microscope (SEM) (JEOL, model JSM-5410LV, Japan) a magnification of 7,000X was used to capture images of whole cell shape and cell wall surface of yeast.

### *The effect of fermented mango wastewater on yeast cell and β-glucan content production*

Finally, the wastewater formulation with the highest yield and β-glucan production was chosen to be further studied in this section. The yield and β-glucan content determination were assessed as previously mentioned. The quality of yeast cells was evaluated for total phenolic compounds, antioxidative properties, and chemical composition. The DPPH scavenging assay and ABTS scavenging assay were analyzed as described below for antioxidative properties. For the chemical composition determination was performed including moister, protein, lipid, fiber, ash, and carbohydrate content (AOAC, 2005). Then, β-glucan was extracted according to the method of Utama *et al.* (2020). Firstly, the yeast cells were collected by using centrifugation at 7,500 rpm for 10 min. The pellets' cell mass (15 %w/v) was immersed in pH 5 distillation conditioned on a 1 M HCl solution and incubated at 50 °C for 48 h. Cell autolysis was done by centrifugation for 10 min at 5,000 rpm. The obtained pellets were dried at 60 °C until extraction. The pellet was washed with 1 M NaOH and mixed for 2 h at 80 °C. The autolyzed cells were recovered by centrifugation for 25 min at 7,500 rpm. The obtained pellet (β-glucan) was dried by using freeze drying at -50 °C. The obtained β-glucan powder was evaluated cell dry weight, β-glucan content, and β-glucan microstructure by using a Scanning Electron Microscope (SEM) (JEOL, model JSM-5410LV, Japan) at a magnification of 2,000X.

### Total phenolic compound determination

The total phenolic content was determined, with a modified method from Iqbal *et al.* (2005). Briefly, 3 g of yeast cell powder mixed with 30 ml of 80% ethanol (v/v). The mixture shakes at 150 rpm for 24 h at room temperature. Then, the supernatant was filtered through Whatman filter paper No. 1. The reaction mixture contained 50 µl of clear soluble, 200 µl of freshly prepared diluted Folin-Ciocalteu reagent from Merck, and 0.5 ml of 7.50% sodium carbonate. The final mixture was diluted to 7 ml with deionized water. The mixtures were kept in the dark at room temperature for 2 h to complete the reaction then the absorbance at 760 nm was determined. The gallic acid was used as the standard. The total phenolic content of the sample was calculated as gallic acid equivalents per g dry weight of extraction. The reaction was conducted in triplicate and results averaged.

### DPPH scavenging assay

The DPPH radical scavenging activity was the ability to reduce the free radical 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH, Sigma). The DPPH radical scavenging activity was modified from the assay method of Gulcin *et al.* (2003). Briefly, 2 ml of the sample solution was mixed with 2 ml of 0.16 mM DPPH in methanol and kept in the dark at room temperature for 30 min to complete the reaction. Next, 2 ml of 80 %v/v methanol was mixed with 2 ml of 0.16 mM DPPH. All sample extracts were kept in the dark at room temperature. The absorbance was measured at 517 nm. The scavenging effect of DPPH free radicals was calculated as follows:

DPPH scavenging activity (%) = $[(A_{control} - A_{sample})/A_{control}]$ x 100

Where $A_{control}$ is the absorbance of the control reaction and $A_{sample}$ is the absorbance of the samples.

### ABTS scavenging assay

The ABTS radical scavenging activities were modified by the assay method of Iqbal *et al.* (2005). To prepare ABTS radical cation, 2.45 mM of potassium persulphate (Merck, Germany) aqueous solution was added with 5 mM ABTS (Sigma-Aldrich, Germany) aqueous solution in equal quantities. The mixtures were kept in the dark at room temperature for 24 h to complete the reaction. Then, 1 ml of the solution was diluted with 60 ml ethanol and used in the ABTS test. The extraction solution (0.10 ml) was added to 2 ml of $ABTS^{+*}$ solution and kept in the dark at room temperature for 10 min to

complete the reaction. The absorbance was measured at 734 nm. The scavenging effect of ABTS free radicals was calculated as follows:

$$\text{Inhibition (\%)} = [(A_{control} - A_{sample})/A_{control}] \times 100$$

## *Data analysis*

Property analysis was carried out in three replicates. The data were subjected to analysis of variance (ANOVA) ($p \leq 0.05$) (Watts *et al*., 1989). Using computer software, Duncan's multiple range test (DMRT) separated the mean with significant differences. For in β-glucan extraction, the mean with significant differences was separated by the Wilcoxon Signed Ranks Test, and the difference was considered statistically significant when the P-value was less than 0.05.

## Results

### *Properties determination fermented mango wastewater*

The properties determination of three types of fermented mango wastewater included physical, chemical, and microbiological were significantly highest lightness (L*) of fermented mango wastewater was exhibited in salt fermented (7.31±0.06) followed by sweet fermented and dried pickled. Nevertheless, the significantly highest greenness (a*) and yellowness (b*) of fermented mango wastewater were found in sweet fermented with the value -2.45±0.05 and 5.62±0.02, respectively. The turbidity of dried pickled showed over evaluation, and salt and sweet fermented exhibited similar data. The color of all fermented mango waste tended to be yellow and green (Table 1). The highest acidity was significantly (≤0.05) for the chemical properties in dried pickled, followed by sweet fermented and salt fermented with the data 1.93±0.02, 2.77±0.02 and 2.86±0.03, respectively. In addition, the significantly highest total soluble solid was found in dried pickled, followed by sweet fermented and salt fermented with the values 66.43±0.50, 15.00±0.00, and 8.00±0.00, respectively. salt content was significantly highest in dried pickled, followed by sweet fermented and salt fermented with the data of 698.20±2.19, 13.00±0.00 and 6.80±0.00 ppt., respectively. It was significantly highest acetic acid content in dried pickled, followed by salt fermented and sweet fermented with the data of 12.41±0.49, 1.00±0.06 and 0.85±0.04%, respectively as presented in Table 2. Microbiological properties were significantly highest in total viable cell count in salt fermented, followed by sweet fermented and dried pickled with the values of $1.66 \times 10^3 \pm 0.45$, $1.43 \times 10 \pm 0.02$ and $<10.00 \pm 0.00$

CFU/ml, respectively. Yeast and mold count in all fermented mango wastewaters indicated as <10.00±0.00 CFU/ml, as presented in Table 3. Then, the optimum conditions of yield and β-glucan production of *S. cerevisiae* TISTR 5059 from three types of fermented mango wastewater were evaluated in the next section.

**Table 1.** Physical properties of three types of fermented mango wastewater

| Fermented mangowastewater types | Colour parameter | | | Terbidity |
|---|---|---|---|---|
| | $L^*$ | a* | b* | |
| Salt fermented | 7.31±0.06[a] | -1.11±0.05[b] | 33.3±0.02[a] | 0.03±0.00[c] |
| Sweet fermented | 5.14±0.02[b] | -24.5±0.05[c] | 5.62±0.02[b] | 0.02±0.00[b] |
| Dried pickled | 0.02±0.01[c] | -0.13±0.01[a] | 0.03±0.01[c] | Over[a] |

L* (lightness) 0 = black, 100 = white
a*(redness/greenness) + = redness, - = greenness
b*(yellowness/blueness) + = yellowness, - = blueness
Each data represents the mean of three replications.
Mean with different letters are statistically different (p≤0.05) according to Duncan's multiple range test.

**Table 2.** Chemical properties of three types of fermented mango wastewater

| Fermented mangowastewater types | pH | Total soluble solid (°Brix) | Salt content (ppt.) | Acetic acid content (%) |
|---|---|---|---|---|
| Salt fermented | 2.86±0.03[a] | 8.00±0.00[c] | 6.80±0.00[c] | 1.00±0.06[b] |
| Sweet fermented | 2.77±0.02[b] | 15.00±0.00[b] | 13.00±0.00[b] | 0.85±0.04[b] |
| Dried pickled | 1.93±0.02[c] | 66.43±0.50[a] | 698.20±2.19[a] | 12.41±0.49[a] |

Mean with different letters are statistically different (ρ≤0.05) according to Duncan's multiple range test.

**Table 3.** Microbiological properties of three types of fermented mango wastewater

| Fermented mangowastewater types | Total viable count (CFU/ml) | Yeast and mold count (CFU/ml)[ns] |
|---|---|---|
| Salt fermented | $1.66 \times 10^3 \pm 0.45^a$ | <10.00±0.00 |
| Sweet fermented | $1.43 \times 10 \pm 0.02^b$ | <10.00±0.00 |
| Dried pickled | <10.00±0.00[c] | <10.00±0.00 |

Mean with different letters are statistically different (ρ≤0.05) according to Duncan's multiple range test.

***Effect of ratio between fermented mango wastewater to wateron yeast growth and β-glucan production***

The effect of the ratio between fermented mango wastewater and water on the yield of yeast cells and β-glucan production were investigated. The 5 ratios of fermented mango wastewater as water including 1:0, 1:1, 1:2, 1:3, and 1:4, were prepared and inoculated with $10^8$ CFU/ml of *S. cerevisiae* TISTR 5059 and cultured at 30 °C for 48 h. Results indicated that increasing the volume of water in the three varieties of fermented mango wastewater resulted in higher cell dry weight and β-glucan production for the yeast cells. Moreover, yeast cells and β-glucan content were significantly highest yield at the ratio of 1:4, followed by 1:3, 1:2, 1:1, and 1:0 respectively in all fermented mango wastewaters. The yeast cell dry weight and β-glucan content were significantly highest in sweet fermented at the ratio of 1:4 with the numeral 1.00±0.02 g/l and 37.90±0.21%w/w, respectively as shown in Figures 1 and 2. Furthermore, the values in yeast cell dry weight and β-glucan content of sweet fermented and salt fermented at the ratio of 1:4 were found in the same trend and higher than the dried pickled. Therefore, in terms of high yeast cell dry weight and β-glucan production, sweet fermented and salt fermented with the ratio of 1:4 was selected to further evaluate the effect of cultivation time on yeast growth and β-glucan production in the next section.
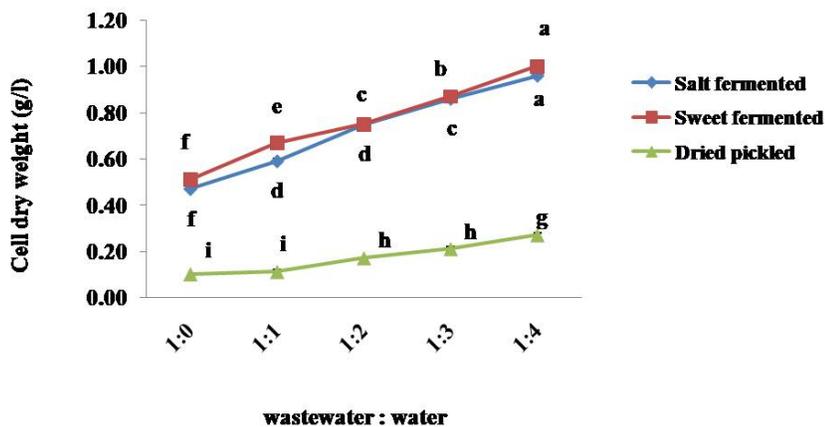


**Figure 1.** Effect of the ratio between fermented mango wastewater and water on yield of yeast cell
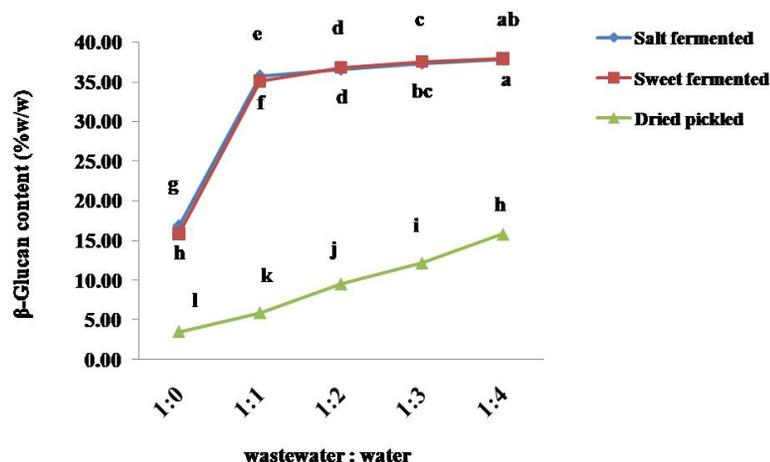
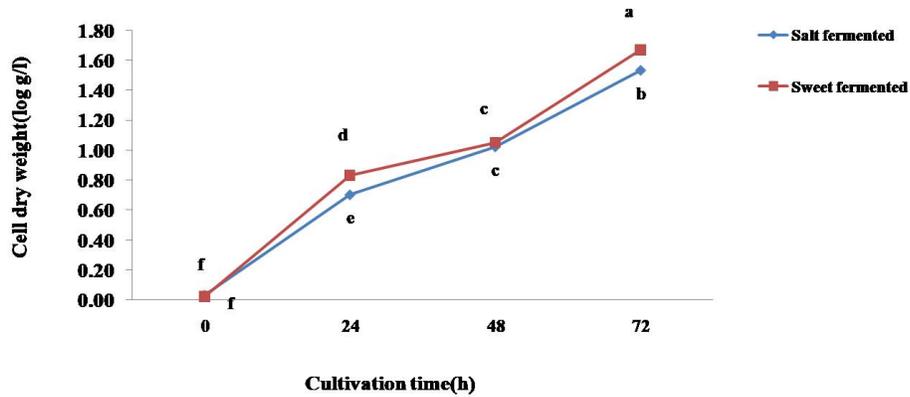**Figure 2.** Effect of the ratio between fermented mango wastewater and water on β-glucan production
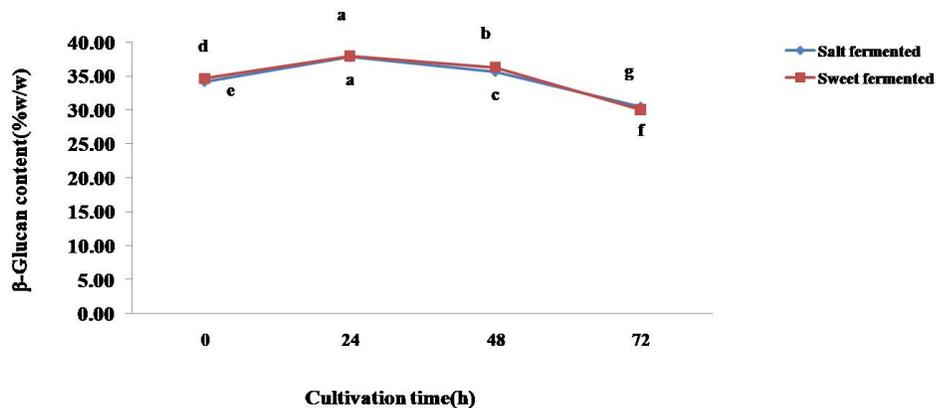
The salt-fermented mango wastewater and sweet-fermented mango wastewater medium with a ratio of 1:4 was determined the cultivation time on yeast growth, β-glucan production, yeast and mold count, and microstructure of yeast cell by using a Scanning Electron Microscope (SEM). Our finding indicated that both salt-fermented and sweet-fermented affected the number of yeast cells and cell dry weight when the cultivation time increased. Additionally, yeast and mold count and yeast cell dry weight were significantly highest in sweet fermented at the ratio of 1:4 for 72 h yeast cultivation with the values of 13.26±0.02 log CFU/ml and 1.67±0.02 g/l, respectively, as shown in Figures3 and 4. In contrast β-glucan content was significantly highest in the yeast cultured in sweet-fermented at the ratio of 1:4 for 24 h cultivation with the value for 37.96±0.18 % w/w. But the value was not significantly differed from in salt-fermented at the ratio of 1:4 for 24 h cultivation with the data for 37.84±0.10 % w/w, as shown in Figure 5.
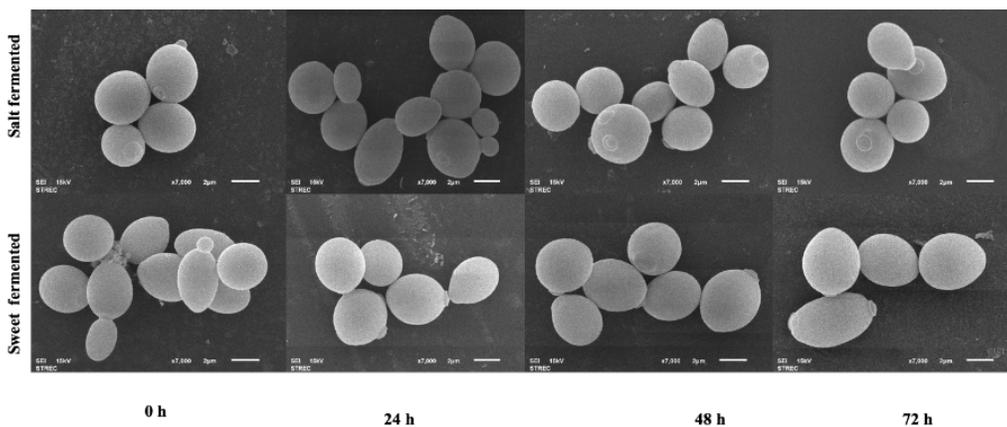
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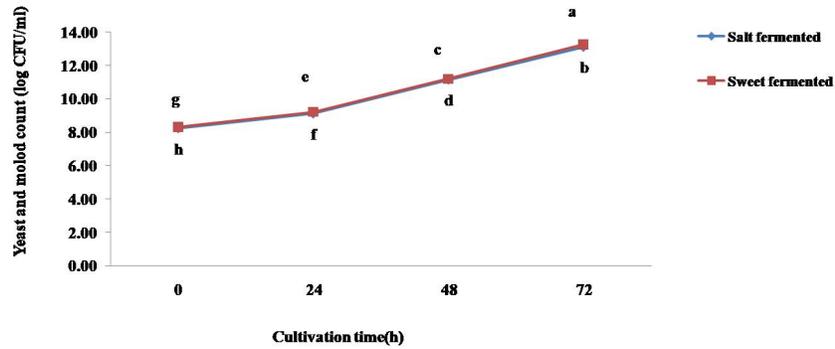
**Figure 3.** Effect of cultivation time on yeast growth comparing salt-fermented and sweet-fermented wastewater medium



**Figure 4.** Effect of cultivation time on the yield of yeast comparing salt-fermented and sweet-fermented wastewater medium

**Figure 5.** Effect of cultivation time on β-glucan production of yeast comparing salt-fermented and sweet-fermented wastewater medium



**Figure 6.** Effect of cultivation time on the microstructure of yeast cell comparing cultured in salt-fermented and sweet-fermented wastewatermedium

    *S. cerevisiae* TISTR 5059 was cultured in fermented mango wastewater the cell morphology by scanning electron microscope (SEM) tended to be broader when increasing the cultivation time (Fig.6). 7,000 magnifications showing the yeast cell had a similar shape based on visual observation, but when comprehensively viewed the shape of the yeast in the salt-fermented tended to be smaller than that of the sweet-fermented at longer cultivation time (72h).

Consequently, fermented mango wastewater effect on some yeast cell properties illustrated that the highest total phenolic compounds were significantly in sweet fermented as the ratio wastewater: water (1:4) for yeast cultivation 72 h with the data of 3.25±0.01 mg/ml. On the other hand, the antioxidative properties were significantly highest in DPPH antioxidant activity and ABTS antioxidant activity which exhibited when yeast was cultivated in the control medium (YPD) with the data of 11.37±0.21% and 106.24±0.65%, respectively as represented in Table 4.

**Table 4.** Effect of fermented mango wastewater on total phenolic compounds and antioxidative activity of yeast cell

| Fermented mango wastewater | Total phenolic compounds | DPPH (%) | ABTS (%) |
|---|---|---|---|
| YPD | 3.15±0.03[b] | 11.37±0.21[a] | 106.24±0.65 [a] |
| 24 h | 3.18±0.01 [b] | 11.09±0.31 [ab] | 104.37±0.32 [b] |
| 72 h | 3.25±0.01[a] | 10.73±0.40 [b] | 98.13±0.32 [c] |

Mean with different letters are statistically different ($\rho \leq 0.05$) according to Duncan's multiple range test.

The fermented mango wastewater effect on the yeast cell's chemical composition showed that the moist and lipid content did not differ significantly between the three cultivation medium conditions. However, the highest protein content (46.42±0.05%) was found in the YPD medium, but it did not significantly differ from the protein content in sweet-fermented cultivation after 24 hours (46.14±0.07%). YPD medium exhibited the highest carbohydrate content of 35.26±0.03%, which was not significantly different from sweet-fermented cultivation for 72 hours at 34.91±0.21% ($p \leq 0.05$). It showed that the highest fiber and ash levels were observed when yeast was fermented in sweet-fermented for 72 hours with values of 7.23±0.01% and 7.44±0.03%, respectively (Table 5).

**Table 5.** The effect of fermented mango wastewater on the chemical composition of yeast cells when compared with cultivation in YPD medium

| Sweet Fermented mango wastewater | Chemical composition±Standard deviation | | | | | |
|---|---|---|---|---|---|---|
| | Moister (%) [ns] | Protein (%) | Lipid (%) [ns] | Fiber (%) | Ash (%) | Carbohydrate (%) |
| YPD | 5.16±0.14 | 46.42±0.05[a] | 0.00±0.00 | 6.12±0.01[c] | .705±0.05 [c] | 35.26±0.03[a] |
| 24 h | 5.12±0.11 | 46.14±0.07[a] | 0.00±.0.00 | 7.10±.0.06 [b] | .718±0.04 [b] | 34.46±0.14[b] |
| 72 h | 5.19±0.06 | 45.24±0.13 [b] | 0.00±.0.00 | 7.23±.0.01 [a] | 7.44±0.03 [a] | 34.91±0.21[ab] |

Mean with different letters are statistically different ($p \leq 0.05$) according to Duncan's multiple range test.
[ns] mean no significant difference ($p \geq 0.05$)

Yield and β-glucan content was significantly highest in sweet-fermented at the ratio wastewater: water (1:4) for yeast cultivation at 72 and 24 h, respectively. Thus, this medium condition was evaluated the effect of fermented mango wastewater on yeast cells and β-glucan determination compared with the control formulation (YPD medium). *S. cerevisiae* TISTR 5059 was cultured in sweet-fermented medium for 24 and 72 h and YPD medium. The β-glucan was extracted from yeast cells using the β-Glucan Assay Kit after collection via centrifugation. The β-glucan content was significantly increased approximately 0.5 times after extraction in all three formulations ($p \leq 0.05$). The highest β-glucan content was found in β-glucan extracted from yeast cultured in YPD medium (control), followed by 24 h cultivation and 72 h in sweet-fermented medium with the values 59.72±0.37, 59.38±0.38 and 58.96±0.44%w/w; respectively as presented in Figure 7.

β-glucan dry weight was significantly lower than yeast cells after extraction of all three medium cultivations. The yield of β-glucan was reduced approximately 5 times in all three formulations (Figure 8).
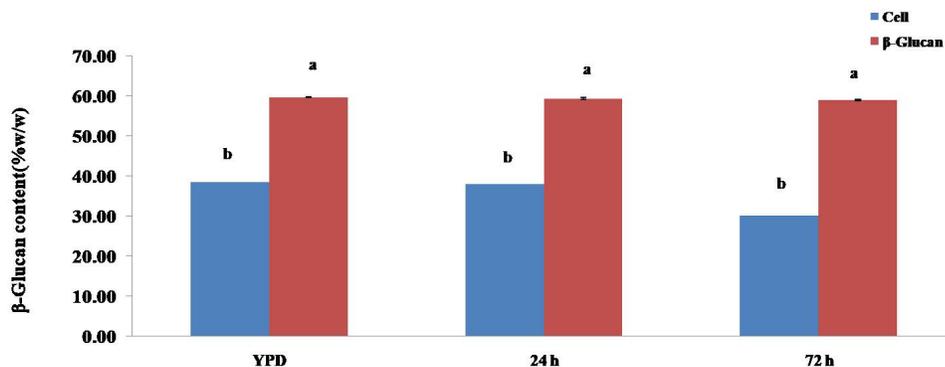


**Figure 7.** Effect of extraction on β-glucan content between YPD medium and sweet-fermented at 24 and 72h

After the yeast cells cultivated in sweet wastewater medium for 24 and 72 h as compared with YPD medium were extracted the β-glucan content and microstructure of yeast cells and β-glucan. The result revealed that the amount of β-glucan content was increased by 0.5 times more than before extraction. In contrast, the yield was lower than before extraction approximately 5 times. The yeast cells were displayed in the cell powder for the microstructure, and the porous powder was performed in β-glucan powder. However, it compared between the yeast cultured in YPD medium and sweet wastewater medium for

24 and 72 h, the microstructure of yeast cell and β-glucan powder were similar, as indicated in Figure 9. Nevertheless, the hole of the β-glucan structure of sweet wastewater for 24 and 72 h cultivation exhibited larger than that control formulation.
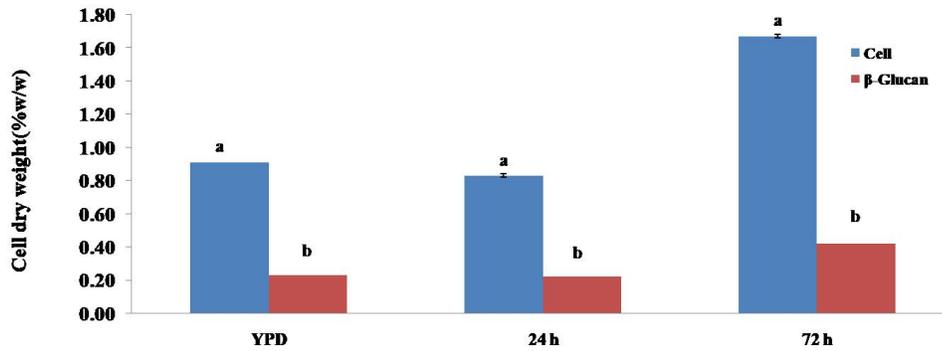


**Figure 8.** Effect of extraction on cell dry weight between YPD medium and sweet-fermented at 24 and 72h
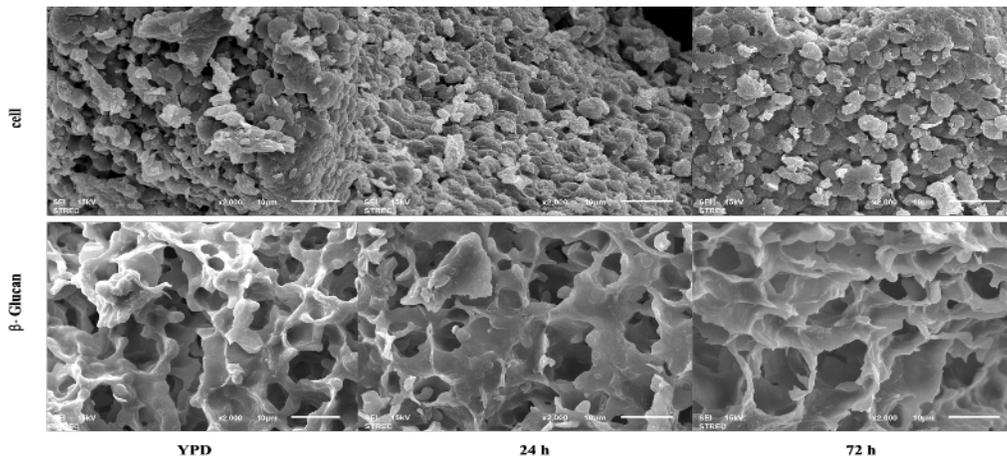


**Figure 9.** SEM of the yeast cell and β-glucan between the yeast cultured in YPD medium and sweet wastewater medium for 24 and 72h

**Discussion**

The properties were determined by three types of fermented mango wastewater: salted-fermented, sweet-fermented, and dried-pickled. In terms of high sugar content in sweet-fermented one was significantly highest in total soluble solids. The optimum condition formulation for the growth of *S. cerevisiae* and β-glucan production, the ratio of wastewater to distilled water at 1:4 showed the highest yield and β-glucan production. Additionally, increasing the ratio of wastewater to water of three types of fermented mango wastewater tended to increase the value of cell dry weight of yeast cells and β-glucan production. It could be due to the condition affected the lowest stress on the yeast cell, and the yeast cell grew faster than other conditions. Then, this condition was studied the optimum time on yield and β-glucan production. Results was significantly highest yield in sweet-fermented yeast cultivation at 72 h and highest β-glucan production was found in sweet-fermented yeast cultivation at 24 h. It could be concerned sugar which is the main nutrient for the yeast growth process (Utama *et al.*, 2020). It is one of the main components that act as an energy producer which is found in the form of glucose, maltose, sucrose, cellulose, hemicelluloses, lactose, and fructose (Kechkar *et al.*, 2019). The best cell wall of *Saccharomyces* sp. contains two outer layers of mannoprotein and an inner β-glucan-chitin layer (Chotigavin *et al.*, 2021). During stress, yeast cells were cultured in the fermented mango wastewater were significantly changed in the cell wall composition. In other words, the β-glucan-chitin layer could be thinner, while the mannoprotein layers become thicker in the osmotic stress condition. Therefore, the β-glucan yield was reduced under the longer cultivation time (Enea *et al.*, 2015). Our result agreed with Varelas *et al.* (2017) who found NaCl and glucose stress impact to reduction of β-glucan formation during alcoholic fermentation. Moreover, exposure of yeast cells to a hyperosmotic solution of NaCl resulted in cell shrinkage and reduced volume, and cell size decreased (Petelenz-Kurdziel *et al.*, 2011).

The fermented mango wastewater on chemical composition of the yeast cell was significantly highest fiber and ash when yeast was cultivated in sweet-fermented for 72 h cultivation. Sweet-fermented has polyphenols and carotenoids which can enter the yeast cell wall via osmosis (García-Mahecha *et al.*, 2023) under stress conditions leading to the repairing cell wall damage occurred (Dewi *et al.*, 2021). Therefore, high mineral and fiber content were observed at 72 h cultivation in the waste formulation.

β-glucan had a similar microstructure but when viewed comprehensively the shape of each β-glucan particle had a different hole size, β-glucan from 72h cultivation showed a larger porous hole than that of the 24 h cultivation and control formulation (Figure 9). The yeast cells were broken in the extraction process, the porous cells were found in β-glucan powder, while the oval yeast

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cells were shown in yeast cells without extraction. It is due to stress or environmental change the cell that must rapidly adjust itself for growth under the new condition by reorganizing the cell wall (Dewi *et al*., 2021). The reorganized cell wall was not strong, and larger holes were found after acid-alkali extraction.

In conclusion, it is suggested that fermented mango wastewater would be used as a medium for producing β-glucan from *S. cerevisiae*. Yeast is cultured in a mixture of water and sweet fermented mango wastewater at a ratio of 1:4 for 24 hours, it produced the best formulation. In addition, this study demonstrated to create value from fermented mango wastewater for yeast waste material that could be exploited to benefit the personal care industry in a sustainable economy. On the other hand, the obtained β-glucan could be evaluated for the biochemical properties for the health-beneficial effect, and for actual production, the production cost and the effect of residual preservatives could be monitored.

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