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Efficacy of different ingredients in solid media on the growth and alcohol production yield of *Saccharomyces cerevisiae*

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Abstract

Yeast is a single-celled fungus that thrives under suitable conditions and is widely used in the production of alcohol and carbon dioxide for various products, such as bread, wine, and other fermented items. In both artisanal and industrial production, selecting the appropriate yeast strain and growth medium is crucial for maximizing profits, particularly when considering cost-effectiveness and ingredient availability. This study aimed to evaluate the effects of different growth media on yeast growth and ethanol production, with a focus on reducing costs and facilitating local sourcing. The experiments were conducted over three months using the streak plate and spread plate methods to determine colony counts. The media compared were: T1 – PDA-Market (commercially purchased), T2 – PDA-Glucose (prepared by adding glucose), and T3 – PDA-Sucrose (prepared by adding sucrose). Colony growth and counts were monitored every 24 hours. This was followed by a yeast fermentation experiment using a 5% sugar solution for 96 hours. The results revealed that PDA-Sucrose (T3) produced the highest number of colonies, with an average of 505.67 ± 4.93 CFU/50 µL. One-way ANOVA analysis showed significant differences in colony growth ($p < 0.01$). However, no significant differences were observed in alcohol yield, biomass, or sugar utilization ($p > 0.05$). Additionally, the PDA-Sucrose medium had the lowest cost, at $0.3423/L. In conclusion, the PDA-Sucrose medium is the best option for yeast production, as it yields a high number of colonies, is easy to source, and is cost-effective. Therefore, PDA-Sucrose media are suitable for both artisanal and industrial alcohol production.

Keywords: Solid media; *Saccharomyces cerevisiae*; Growth; Alcohol; Production yield

1. Introduction

Yeast (*Saccharomyces cerevisiae*) is a unicellular microorganism widely utilized across various industries, particularly in the fermentation process for converting sugar into alcohol and carbon dioxide (Rutz & Janssen, 2007). Among the many yeast strains, *S. cerevisiae* is especially favored in ethanol production due to its ability to thrive in high sugar concentrations, ranging from 12% to 20% (Tamanag, 2010). This yeast plays a crucial role in advancing food processing, biotechnology, and numerous other industrial applications (Johnson & Echavarri-Erasun, 2011). When selecting yeast strains for fermentation, key factors such as growth rate, ethanol yield, and the production of flavor compounds are typically considered (Briggs et al., 2004). The efficiency of fermentation largely depends on the appropriate selection of yeast strains, the use of suitable growth media, and the implementation of effective sanitation practices (Sloley, 2001). While dry yeast is convenient for storage and transportation, it requires rehydration prior to use in fermentation processes (Slaa & Else, 2009). Sugar types such as glucose and sucrose play essential roles in the metabolism of *S.*

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cerevisiae (Verstepen et al., 2004; Sota & Tetsuo, 2011). Verstepen et al. (2004) highlighted that both glucose and sucrose can be directly utilized by *S. cerevisiae* for ethanol production. However, the choice of sugar in growth media significantly influences fermentation efficiency (Parapouli et al., 2020; Vejarano & Gil-Calderón, 2021). In Cambodia, local wine production is well-established, yet producers face challenges with pricing and difficulties sourcing raw materials (Chakrya, 2018). In Kratie province, only 10% of the 761 registered wine-producing households have adopted standardized practices, contributing to a high incidence of alcohol poisoning cases (Chakrya, 2018). Chay et al. (2017) and Serio et al. (2003) emphasized that selecting appropriate raw materials can reduce contamination and improve ethanol yield. Thompson et al. (2021) noted that food safety awareness remains low in Cambodia, particularly in wine production. In alcohol production, the quality of raw materials is crucial in determining both product quality and cost-effectiveness (Fiore et al., 2020; Jiao et al., 2017).

Given these challenges, the present study aims to explore the use of different growth media for yeast cultivation and assess their impact on ethanol yield.

2. Materials and methods

2.1. Strain, media and broth

The *S. cerevisiae* strain was obtained from the Food Processing Laboratory at the University of Kratie and was re-streaked onto fresh potato dextrose agar (PDA) to isolate and obtain new colonies. Three types of PDA media were prepared for the experiment. PDA-Market was prepared by dissolving 40 grams of commercial PDA powder in 1 liter of reverse osmosis (RO) water. PDA-Glucose was prepared by peeling and weighing 200 grams of potatoes, boiling them in RO water for 10 minutes, filtering the solution, and adjusting the volume to 1 liter with RO water. Then, 20 grams of glucose and 15 grams of agar were added. PDA-Sucrose was prepared following the same procedure as PDA-Glucose, except that glucose was replaced with 20 grams of sucrose. All media types were sterilized by autoclaving at 121°C and 106 kPa (1 atm) for 20 minutes in flasks. After sterilization, the media were poured into sterile plates and labeled according to the type of medium they contained. For fermentation, a 5% (w/v) sucrose broth was prepared by dissolving 5 grams of sucrose in 100 mL of RO water in a 250 mL flask, which was then autoclaved.

2.2. Yeast incubation

The newly isolated *S. cerevisiae* strain was streaked onto each type of prepared medium. The plates were incubated at 37°C for 24 hours to allow for colony formation. After incubation, colonies from each medium were subjected to serial dilution and spread onto fresh media to determine the colony-forming units (CFU). All plates were then incubated at 37°C for an additional 72 hours to allow for the development of countable colonies. The colonies were quantified using a Dot Colony Counter (Model: DOT 120 mm Magnifying Glass).

2.3. Experimental design and treatments

The experiment was designed using a completely randomized design, ensuring homogeneous conditions across all treatments and replications. A total of three treatments were included, each replicated five times. The treatments were as follows:

- T1: PDA medium prepared using commercial PDA powder.
- T2: PDA medium prepared with potato extract, glucose, and agar powder.
- T3: PDA medium prepared with potato extract, sucrose, and agar powder.

2.4. Batch fermentation

Each *S. cerevisiae* colony, isolated from solid media, was transferred into a 250 mL flask containing 100 mL of a 5% (w/v) sucrose solution. The strains from the different media were cultured in the same broth for batch fermentation. The flasks were sealed and incubated on an IKA Shaker (Model: KS 260 Basic, No: 0002980200) at 200 rpm and room temperature. Samples were collected daily over a 96-hour period to analyze sugar content, alcohol production, and biomass (In et al., 2020; Sokra et al., 2024c).

2.5. Sugar content and biomass measurement

Sugar content was measured using a Brix refractometer (Model: RHB-32ATC, Brix range: 0-32%), and alcohol content was determined using an alcohol refractometer (Alcohol range: 0-50% v/v). For both measurements, three drops of each sample were placed onto the refractometer's prism, and the corresponding sugar or alcohol levels were recorded

from the scale. Biomass was assessed by collecting 1 mL of the sample daily, followed by centrifugation for 1 minute. The supernatant was then used for measuring sugar and alcohol content, while the cell pellet was weighed using an electronic balance (Model: AUW-D) (Sokra et al., 2024b).

2.6. Data analysis

All data were initially entered into Microsoft Excel Professional Plus 2021 for cleaning and organization. Statistical analysis was conducted using GraphPad Prism software (Version 10.2.0, Windows, San Diego, California, USA; www.graphpad.com). The results are presented as means ± standard deviations (SDs). Differences between treatment means were evaluated using one-way ANOVA, followed by Tukey's HSD multiple comparisons test. The significance level for all tests was set at $p < 0.01$ or $p < 0.05$.

3. Results

3.1. Number and morphology of colonies

After 72 hours of incubation, the number of colonies on the plates varied based on the type of growth medium used. Figure 1 illustrates the differences in colony formation after inoculating the yeast strain onto PDA plates with various growth media. The colonies grown on PDA-Market were the largest, followed by those on PDA-Glucose, which were of medium size. The colonies on PDA-Sucrose were the smallest compared to those on PDA-Market and PDA-Glucose.

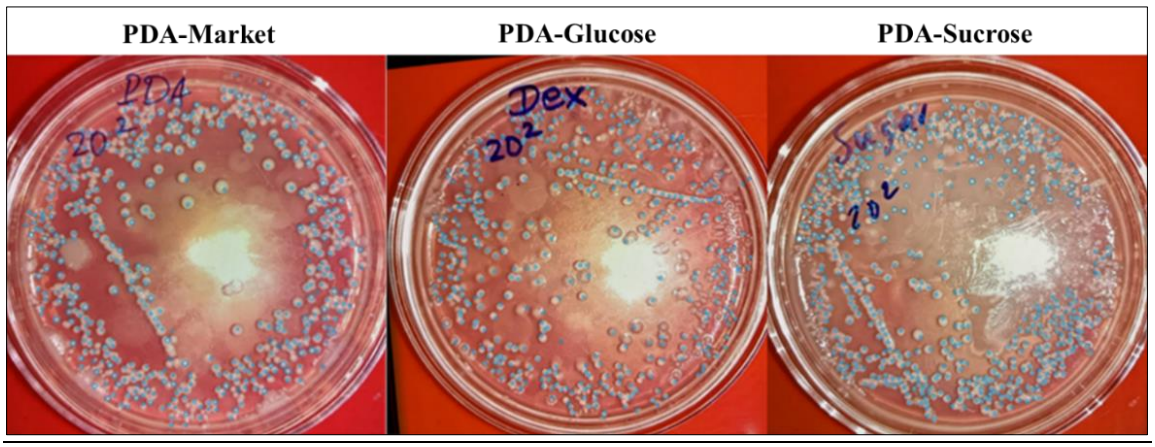

Figure 1 Amount and morphology of *S. cerevisiae* colonies on different type of media

Table 1 Comparing colonies in each type of media

Treatments	Means differences	p-value
T1 vs. T2	17.67	0.0092
T1 vs. T3	-71.67	0.0001
T2 vs. T3	-89.33	0.0001

Table 1 shows the mean differences in colony counts for each treatment. PDA-Market and PDA-Glucose are significantly different ($p < 0.05$), while PDA-Market and PDA-Sucrose, as well as PDA-Glucose and PDA-Sucrose, are significantly different ($p < 0.01$).

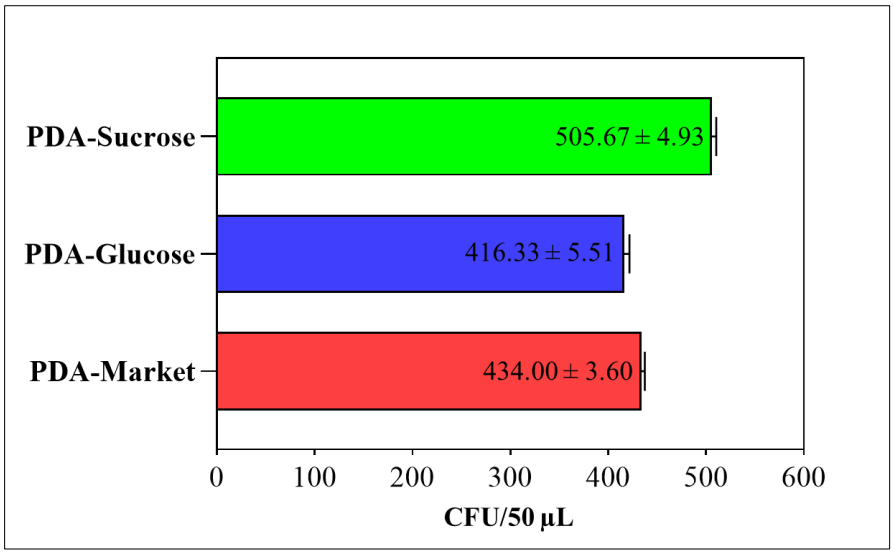

Figure 2 Amounts of colonies forming unit in different type of media on plates**.**

The results, shown in **Figure 2**, revealed significant differences in colony counts after 72 hours of incubation on all media types. PDA-Sucrose yielded the highest average number of colonies, with 505.67 ± 4.93 CFU/50 µL, followed by PDA-Market with 434.00 ± 3.60 CFU/50 µL, and PDA-Glucose with the lowest at 416.33 ± 5.51 CFU/50 µL. Statistical analysis using one-way ANOVA confirmed that the differences in colony counts were statistically significant ($p < 0.01$).

3.2. Production of alcohol from batch fermentation

Figure 3 presents the growth rate of *S. cerevisiae*, sugar utilization, and alcohol production across different types of media. All strains, regardless of the medium, completely consumed sucrose within 4 days. The strains from PDA-Market and PDA-Sucrose produced higher biomass, with an average of 0.12 g/mL, compared to the PDA-Glucose strain, which had an average biomass of 0.11 g/mL. Alcohol production was consistent across all strains, with an average yield of 4.1%.

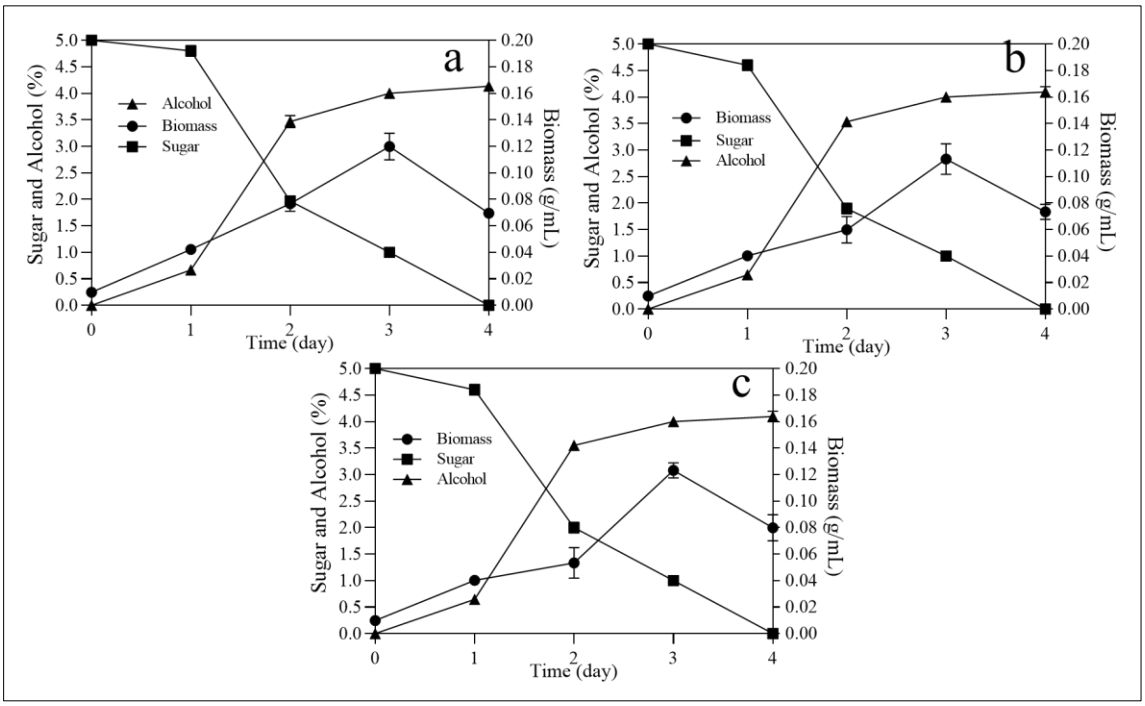

Figure 3 Batch fermentation over 96 hours with 5% (w/v) sucrose. (a) *S. cerevisiae* isolated from PDA-Market, (b) *S. cerevisiae* isolated from PDA-Glucose, and (c) *S. cerevisiae* isolated from PDA-Sucrose.

Statistical analysis of sugar utilization, biomass, and alcohol production revealed no significant differences ($p > 0.05$). The main finding from these results is that the different types of media used in this experiment did not have a significant impact on the growth of *S. cerevisiae* or on the alcohol yield.

3.3. Raw material expenses for teach medium per liter

Table 2 Comparing total expense in each medium

PDA-Market				
No.	**Raw material**	**Price**	**Usage**	**Total price**
1	PDA-commercial powder	0.18$/g	40 g	7.2$
2	RO water	0.0049$/L	1L	0.0049$
Total				7.2049$
PDA-Glucose				
1	Potatoes	1.36$/Kg	200 g	0.272$
2	Glucose	29.64$/Kg	20 g	0.5928$
3	RO water	0.0049 $/L	1 L	0.0049$
4	Agar powder	0.86$/Kg	30 g	0.0258$
Total				0.8955$
PDA-Sucrose				
1	Potatoes	1.36$/Kg	200 g	0.272$
2	Sucrose	1.98$/Kg	20 g	0.0396$
3	RO water	0.0049 $/L	1 L	0.0049$
4	Agar powder	0.86$/Kg	30 g	0.0258$
Total				0.3423$

To prepare the media, various raw materials were utilized, and the associated costs for preparing PDA-Market, PDA-Glucose, and PDA-Sucrose were found to be \$7.20/L, \$0.90/L, and \$0.34/L, respectively (Table 2). A comparison of these costs revealed that PDA-Market was approximately 8 times more expensive than PDA-Glucose and 21 times more expensive than PDA-Sucrose. Additionally, PDA-Glucose was 3 times more costly than PDA-Sucrose. The primary conclusion drawn from this experiment is that PDA-Sucrose offers the most cost-effective option for preparing *S. cerevisiae* cultures.

4. Discussion

4.1. Number and morphology of colonies

This study revealed that incubating *S. cerevisiae* on different media resulted in varying colony sizes and morphologies. These findings align with Mwesigye and Barford (1996), who suggested that yeast hydrolyzes carbohydrates to produce energy for growth and binary fission. However, Sokra et al. (2024a) and In et al. (2020) demonstrated that larger colonies were not ideal for selection, as they exhibited lower capacities to produce by-products and secondary metabolites. Consequently, PDA-Glucose and PDA-Sucrose media exhibited more favorable morphologies for subsequent fermentation processes. These results are consistent with Ly et al. (2018), who reported that *S. cerevisiae* can adapt to sucrose and utilize it directly as a primary sugar, similarly to glucose. *S. cerevisiae* grown on PDA-Sucrose produced the highest colony counts, corroborating the findings of Marques et al. (2015) and Smith and Dayton (1974), who observed that sucrose adaptation significantly increased colony numbers compared to media lacking sugars. Additionally, Pepin and Marzzacco (2015) found that sucrose in the growth medium enhanced *S. cerevisiae* cell proliferation more rapidly than other sugars. PDA-Malt and PDA-Glucose also supported robust *S. cerevisiae* growth, in agreement with the work of Moore et al. (2021) and Otterstedt et al. (2004), who emphasized glucose as a crucial monosaccharide for yeast metabolism, growth, and reproduction.

4.2. Batch fermentation of *S. cerevisiae* from different media

The batch fermentation of *S. cerevisiae* on various types of media with 5% sucrose was conducted to produce ethanol and evaluate the biological morphologies following the utilization of different growth media. This study found no significant differences in biomass production, ethanol yield, or sugar utilization across the tested media. These results are consistent with Lee (2023), who reported that sucrose and glucose did not influence colony morphology during ethanol production, underscoring their suitability and cost-effectiveness as substrates for further applications. In agreement with these findings, Lee and Kim (2016) observed that sucrose is hydrolyzed into glucose and fructose, which are subsequently converted into pyruvate for energy generation. In contrast, glucose, a monosaccharide, is directly utilized by yeast cells for energy production and fermentation under anaerobic conditions (Christiansen et al., 2018; Moore et al., 2021). These results indicate that both sucrose and glucose follow similar metabolic pathways, being converted into pyruvate, which accounts for the lack of significant differences in growth, ethanol production, or sugar utilization. Furthermore, this study supports the findings of Wilson et al. (2019), who demonstrated that the metabolism of sucrose and glucose in yeast resulted in comparable growth performance.

4.3. Comparing raw material expense

The study demonstrated that PDA-Sucrose had the lowest raw material costs while yielding the highest colony counts. These findings suggest that sucrose-based growth media are highly effective in promoting yeast cell proliferation. Specifically, the cost of PDA-Sucrose was 21 times lower than PDA-Market and 3 times lower than PDA-Glucose. This aligns with the findings of Hubalek et al. (2022), who emphasized that raw material costs are a crucial factor in reducing the overall expenses of growth media. Similarly, Black (2020) noted that while commercially available PDA growth media offer convenience, they tend to be more expensive than self-prepared alternatives. Furthermore, Dranka et al. (2020) demonstrated that utilizing readily available and cost-effective raw materials enhances the scalability of production, which is consistent with the results of this study.

5. Conclusion

S. cerevisiae was streaked onto different types of fresh solid media to obtain countable colonies, which were subsequently sub-cultured for growth, yielding a substantial number of colonies. Among the tested media, PDA-Sucrose (T3) produced the highest colony count, averaging 505.67 ± 4.93 CFU/50 µL, with favorable colony size and morphology. Batch fermentation in a 5% sucrose solution revealed no significant differences in sugar utilization, alcohol production, or biomass across the conditions. PDA-Sucrose demonstrated the lowest raw material cost at $0.3423/L, which was 21 times lower than PDA-Market and 3 times lower than PDA-Glucose. These findings emphasize the cost-effectiveness and growth-promoting potential of PDA-Sucrose as an efficient growth medium.

However, this study has certain limitations. It focused solely on three growth media, which may not encompass other potential media options. The experiments were conducted in a laboratory setting, which may not accurately represent large-scale industrial conditions, and only one yeast strain (*S. cerevisiae*) was tested. Future research should explore a broader range of growth media and yeast strains, optimize fermentation conditions, and incorporate advanced analytical methods to enhance the study's applicability to industrial processes. Additionally, further cost analysis could offer more profound insights into the scalability and practical implementation of these findings.

Compliance with ethical standards

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Disclosure of conflict of interest

Each author declares that he/she has no conflict of interest.

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